Vaccine xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Efficacy of inactivated swine influenza virus vaccines against the 2009 A/H1N1 influenza virus in pigs

Amy L. Vincent[a,*], Janice R. Ciacci-Zanella[a,b], Alessio Lorusso[a], Philip C. Gauger[c], Eraldo L. Zanella[a,d], Marcus E. Kehrli Jr.[a], Bruce H. Janke[c], Kelly M. Lager[a]

[a] Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA
[b] Labex-USA, EMBRAPA – Brazilian Agriculture Research Corporation, Brasilia, DF, Brazil
[c] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[d] Faculdade de Agronomia e Medicina Veterinaria, Universidade de Passo Fundo, Campus 1, Bairro São José, Passo Fundo, RS, Brazil

ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 18 January 2010
Accepted 20 January 2010
Available online xxx

Keywords:
A/H1N1 2009
Swine
Influenza A virus
Vaccine

ABSTRACT

The gene constellation of the 2009 pandemic A/H1N1 virus is a unique combination from swine influenza A viruses (SIV) of North American and Eurasian lineages, but prior to April 2009 had never before been identified in swine or other species. Although its hemagglutinin gene is related to North American H1 SIV, it is unknown if vaccines currently used in U.S. swine would cross-protect against infection with the pandemic A/H1N1. The objective of this study was to evaluate the efficacy of inactivated vaccines prepared with North American swine influenza viruses as well as an experimental homologous A/H1N1 vaccine to prevent infection and disease from 2009 pandemic A/H1N1. All vaccines tested provided partial protection ranging from reduction of pneumonia lesions to significant reduction in virus replication in the lung and nose. The multivalent vaccines demonstrated partial protection; however, none was able to prevent all nasal shedding or clinical disease. An experimental homologous 2009 A/H1N1 monovalent vaccine provided optimal protection with no virus detected from nose or lung at any time point in addition to amelioration of clinical disease. Based on cross-protection demonstrated with the vaccines evaluated in this study, the U.S. swine herd likely has significant immunity to the 2009 A/H1N1 from prior vaccination or natural exposure. However, consideration should be given for development of monovalent homologous vaccines to best protect the swine population thus limiting shedding and the potential transmission of 2009 A/H1N1 from pigs to people.

Published by Elsevier Ltd.

1. Introduction

Since 1997–98 multiple subtypes of endemic SIV, H3N2, H1N1, and H1N2 with a triple reassortant internal gene (TRIG) constellation derived from swine, avian and human influenza viruses, co-circulate in most major swine producing regions of the U.S. and Canada (reviewed in Ref. [1]). Additionally, introduction of H1N1 and H1N2 viruses with the HA and NA genes originating from contemporary human seasonal influenza A viruses (hu-like H1) that are genetically and antigenically distinct from the classical swine H1 lineage were reported in pigs in Canada [2]. The viruses identified in Canadian pigs were human lineage in entirety or double (human–swine) reassortants. Since 2005, hu-like H1N1 and H1N2 viruses have emerged in swine herds across the U.S. as human–swine reassortants possessing the TRIG (H1 δ-cluster) [3]. As a result of these interspecies reassortment events and antigenic drift, 4 phylogenetic clusters (α, β, γ, and δ) of H1 SIV are now endemic in U.S. swine [3,4]. Eurasian lineage SIVs have not been reported to circulate in U.S. swine.

In March–April 2009, a novel pandemic A/H1N1 emerged in the human population in North America [5]. The gene constellation of the emerging virus was demonstrated to be a combination of genes from swine influenza A viruses (SIV) of North American and Eurasian lineages that had never before been identified in swine or other species. The emergent A/H1N1 quickly spread in the human population and the outbreak reached pandemic level 6 as declared by the World Health Organization on June 11, 2009. Although the 8 gene segments of the novel virus have lineages with available sequences of corresponding genes from SIV from North America or Eurasia, no closely related ancestral SIV with this gene combination has been identified in North America or elsewhere in the world [6,7]. Other than sporadic transmission to humans [8,9], swine influenza A viruses of the H1N1 subtype historically have been distinct from avian and other mammalian H1N1 influenza viruses in

* Corresponding author at: Virus and Prion Diseases Research Unit, National Animal Disease Center, USDA-ARS, 1920 Dayton Road, Ames, IA 50010, USA. Tel.: +1 515 337 7557; fax: +1 515 337 7428.
E-mail address: amy.vincent@ars.usda.gov (A.L. Vincent).

0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2010.01.049

characteristics of host specificity, serologic cross-reactivity, and/or nucleotide sequence. Although the HA gene from the 2009 pandemic A/H1N1 has ancestry with the γ-cluster H1 SIV from North America, it is unknown whether prior immunity to endemic SIV or SIV vaccines would cross-protect against the 2009 pandemic A/H1N1.

Vaccines against swine influenza virus (SIV) are commonly used in the U.S. swine industry. Most fully licensed vaccines are multivalent in nature with H1 and H3 subtypes included. In recent years, use of autogenous vaccines [10] has become increasingly widespread in an attempt to address the dynamic nature of SIV in the U.S. In 2008, over half of the doses of SIV vaccine released for sale were autogenous (Dr. Rick Hill, USDA-APHIS–Center for Veterinary Biologics, personal communication). These vaccines require basic purity and safety testing, but not the rigorous purity, safety, potency and efficacy testing required of fully licensed SIV vaccines; therefore they can have only limited distribution in that they must only be used in the herd of origin. Most autogenous vaccines are also multivalent with various combinations of H1 and H3 subtypes.

In this study, weaned pigs were vaccinated with 4 different inactivated vaccines produced from endemic North American SIV. Following 2 doses of vaccine, pigs were challenged with a 2009 A/H1N1 virus to evaluate the effectiveness of the vaccines to reduce virus shedding, pneumonia, and clinical disease. Significant protection from virus replication and shedding was demonstrated for 3 of the vaccines. In particular, the homologous monovalent vaccine induced optimal protection by complete prevention of viral replication in the nasal epithelium and lungs as well as significant reduction in pneumonia and febrile response to challenge with 2009 A/H1N1. Strong serologic cross-reactivity with a γ-cluster H1 SIV was demonstrated with 2009 A/H1N1 vaccine antisera, suggesting robust titers against γ-cluster H1 SIV may be correlated with partial cross-protection against pandemic A/H1N1.

2. Materials and methods

2.1. In vivo vaccine study

Sixty three-week old cross-bred pigs were obtained from a herd free of SIV and porcine reproductive and respiratory syndrome virus (PRRSV) and treated with ceftiofur crystalline free acid according to label (Pfizer Animal Health, New York, NY) to reduce bacterial contaminants prior to the start of the study. Pigs were housed in biosafety level 2 (BSL2) containment during the vaccine phase of the study. On the day of challenge, pigs were transferred to ABSL3 containment for the remainder of the experiment. Pigs were cared for in compliance with the Institutional Animal Care and Use Committee of the National Animal Disease Center. A/California/04/2009 H1N1 (CA/09) (cell passage 1) was received from the Centers for Disease Control and Prevention (CDC) and propagated in Madin-Darby Canine Kidney (MDCK) cells for use in the studies described below. Three inactivated vaccines (Vaccines A, B, and D) were selected based on serologic evidence of cross-reactivity in a previous study using vaccine antisera supplied by commercial vaccine manufacturers [11]. Commercial vaccines were supplied by the manufacturers for use in the study and administered as per label or manufacturer recommendation. Vaccine A (FluSure® XP, Pfizer Animal Health, New York, NY) is a fully licensed trivalent commercial product containing cluster IV H3N2, γ-cluster H1N1, and δ-cluster H1N1 SIV as vaccine seed viruses. Vaccine B (MaxiVac Excell® 5.0, Intervet/Schering-Plough, Boxmeer, The Netherlands) is a pentavalent product under review for full licensure containing clusters I and IV H3N2 and β-, γ-, and δ-cluster H1 SIV as vaccine seed viruses. Vaccine D (Newport Labs, Worthington, MN) is a bivalent autogenous vaccine containing β- and γ-cluster H1 SIV as vaccine seed viruses. A monovalent experimental vaccine (Vaccine E) was prepared from CA/09 at 8 HA units per 50 μL and $1 \times 10^{6.5}$ 50% tissue culture infectious dose ($TCID_{50}$) per mL with inactivation by ultraviolet irradiation and addition of a commercial adjuvant (Emulsigen D, MVP Labs) at a v:v ratio of 4:1 virus to adjuvant. Pigs ($N = 10$ per group) were vaccinated with 2 mL of each vaccine by the intramuscular route at approximately 4 weeks of age, boosted at 7 weeks of age, and challenged at 10 weeks of age.

For viral challenge, pigs were inoculated intratracheally with 2 mL of 1×10^5 $TCID_{50}$ of CA/09 as previously described [4]. Pigs were observed daily for signs of clinical disease and fever. Nasal swabs (Fisherbrand Dacron swabs, Fisher Scientific, Pittsburg, PA) were taken on 0, 3, and 5 days post-infection (dpi) to evaluate nasal virus shedding by dipping the swab in minimal essential medium (MEM) and inserting the swab approximately 2.5 cm into each nares. Swabs were then placed into 2 mL MEM and stored at −80° C until study completion. Pigs were humanely euthanized with a lethal dose of pentobarbital (Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA) on 5 dpi to evaluate lung lesions and viral load in the lungs.

2.2. Pathologic examination of lungs

At necropsy, lungs were removed and evaluated for the percentage of the lung affected with plum colored and well-demarcated consolidated lesions typical of influenza virus infection in pigs. The percentage of the surface affected with pneumonia was visually estimated for each lung lobe, and a total percentage for the entire lung was calculated based on weighted proportions of each lobe to the total lung volume as previously described [12]. Each lung was then lavaged with 50 mL MEM to obtain bronchoalveolar lavage fluid (BALF). Tissue samples from the trachea and right cardiac lung lobe and other affected lobes were taken and fixed in 10% buffered formalin for histopathologic examination. Tissues were routinely processed and stained with hematoxylin and eosin. Lung sections were given a score from 0 to 3 to reflect the severity of bronchial epithelial injury using previously described methods [13].

2.3. Diagnostic microbiology

All pigs were screened for influenza A nucleoprotein antibody by ELISA (MultiS ELISA, IDEXX, Westbrook, Maine) prior to the start of the study to ensure absence of prior immunity. BALF samples from 5 dpi were screened for aerobic bacterial growth on blood agar and Casmin (NAD enriched) plates. Diagnostic PCR for PCV2 [14] and *Mycoplasma hyopneumoniae* [15] or an in-house RT-PCR for PRRSV was conducted on nucleic acid extracts from BALF.

2.4. Viral replication and shedding

Nasal swab samples were subsequently thawed and vortexed for 15 s, centrifuged for 10 min at 640 × *g* and the supernatant was passed through 0.45 μm filters to reduce bacterial contaminants. Ten-fold serial dilutions in serum-free MEM supplemented with TPCK trypsin and antibiotics were made with each BALF sample and nasal swab filtrate sample. Each dilution was plated in triplicate in 100 μL volumes onto PBS-washed confluent MDCK cells in 96-well plates. Plates were evaluated for CPE between 48–72 h post-infection. At 72 h, plates were fixed with 4% phosphate-buffered formalin and stained using immunocytochemistry with an anti-influenza A nucleoprotein monoclonal antibody as previously described [16]. A $TCID_{50}$ titer was calculated for each sample using the method of Reed and Muench [17].

Please cite this article in press as: Vincent AL, et al. Efficacy of inactivated swine influenza virus vaccines against the 2009 A/H1N1 influenza virus in pigs. Vaccine (2010), doi:10.1016/j.vaccine.2010.01.049

2.5. Serologic assays

For use in the hemagglutination inhibition (HI) assay, sera were heat inactivated at 56 °C for 30 min, then treated to remove non-specific hemagglutinin inhibitors and natural serum agglutinins by treatment with a 20% suspension of kaolin (Sigma Aldrich, St. Louis, MO) and adsorption with 0.5% turkey red blood cells (RBC). The HI assays were then performed with CA/09 and A/Mexico/4108/2009 (MX/09) pandemic A/H1N1, A/Swine/MN/37866/1999 H1N1 (MN/99; α-cluster SIV), A/Swine/NE/2013/2008 H1N1 (NE/08; β-cluster SIV), and A/Swine/OH/51145/2007 H1N1 (OH/07; γ-cluster SIV) viruses as antigens and turkey RBC using standard techniques [18]. Log_2 transformations were analyzed and geometric mean reciprocal titers reported.

ELISA assays to detect total IgG and IgA antibodies specific to 2009 pandemic H1N1 present in serum and BALF were performed as previously described [19] with modifications. Concentrated A/California/07/2009 New York Medical Center reassortant (X-179A) virus was resuspended in Tris–EDTA basic buffer, pH 7.8, and diluted to an HA concentration of 100 HA units/50 μL. Immulon-2HB 96-well plates (Dynex, Chantilly, VA) were coated with 100 μL of antigen solution and incubated at room temperature overnight. Serum was diluted 1000-fold followed by 2-fold serial dilutions in PBS. BALF was diluted by 2-fold serial dilutions in MEM. The assays were performed on each sample in duplicate. The mean of duplicate wells was calculated and antibody titers were designated as the highest dilution with an OD greater than 2 standard deviations above the mean of the non-vaccinated, non-challenged negative controls. Log_2 transformations were analyzed and geometric mean reciprocal titers are reported.

2.6. Statistical analysis

Macroscopic pneumonia scores, microscopic pneumonia scores, log_{10} transformed BALF and nasal swab virus titers, and log_2 transformations of HI reciprocal titers or ELISA reciprocal titers were analyzed using analysis of variance (ANOVA) with a P-value ≤ 0.05 considered significant (GraphPad Prism, GraphPad Software, La Jolla, CA). Response variables shown to have a significant effect by treatment group were subjected to pair-wise comparisons using the Tukey–Kramer test. Rectal temperature data were analyzed using a mixed linear model for repeated measures (SAS, SAS Institute, Cary, NC). Linear combinations of the least squares means estimates for rectal temperatures were used in a priori contrasts after testing for a significant treatment group effect ($P < 0.05$). Comparisons were made between each treatment group at each time point using a 5% level of significance ($P < 0.05$) to assess statistical differences.

3. Results

3.1. Inactivated vaccines reduced CA/09 virus levels and clinical disease

All pigs were free of influenza A virus and influenza A virus antibodies prior to the start of the experiment. All pigs were negative for extraneous viral and *M. hyopneumoniae* in the BALF at 5 dpi. All pigs except one were negative for significant aerobic bacterial growth at necropsy. A single pig had significant colony counts of a *Streptococcus* species in the BALF. Two doses of the homologous CA/09 vaccine prevented nasal shedding in all pigs challenged with CA/09 A/H1N1 at both time points after challenge (Table 1). In addition, infectious virus was not detected from the lungs of any pigs vaccinated with the homologous vaccine. There were statistically significant reductions in group mean virus titer levels in the A and D vaccine groups in nasal swabs at 3 and 5 dpi and in BALF at 5 dpi. However, both groups exhibited detection of virus in the nose or lung.

Table 1
Virus titers in nasal swabs and BALF.[a].

Group	Nasal swabs		BALF
	3 dpi	5 dpi	5 dpi
Vaccine A	0.1 ± 0.1/1	0.4 ± 0.3/3*	1.3 ± 0.6/4*
Vaccine B	0.8 ± 0.3/5	3.0 ± 0.3/10	2.9 ± 0.7/9
Vaccine D	0.0 ± 0.0/0	0.3 ± 0.3/2*	0.3 ± 0.2/2*
Vaccine E	0.0 ± 0.0/0	0.0 ± 0.0/0*	0.0 ± 0.0/0*
NV/Challenge	0.5 ± 0.3/4	2.7 ± 0.2/10	4.3 ± 0.2/10
NV/NC	0.0 ± 0.0/0	0.0 ± 0.0/0*	0.0 ± 0.0/0*

Vaccine A, fully licensed trivalent vaccine; Vaccine B, fully licensed pentavalent vaccine; Vaccine D, bivalent autogenous vaccine; Vaccine E, monovalent experimental CA/09 vaccine; NV/Challenge, non-vaccinated, challenged positive control group; NV/NC, non-vaccinated, non-challenged negative control group.

[a] Log_{10} geometric mean titer ± standard error of the mean/number positive out of 10 per group. BALF, bronchoalveolar lavage fluid; dpi, days post-infection.

* Significantly different from NV/Challenge control group at $P < 0.05$.

Macroscopic pneumonia in the non-vaccinated challenge control group was typical of influenza induced lesions in a 10-week old pig (Table 2). All vaccine groups demonstrated statistically significant reduction in percentage of macroscopic pneumonia over the positive controls. Additionally, the E and D vaccines were statistically indistinguishable from the non-challenged negative controls. Microscopic changes associated with influenza infection in the lungs were reflective of the macroscopic pneumonia (Table 2).

Pigs challenged intratracheally with the CA/09 H1N1 pandemic virus had a significant ($P < 0.0001$) febrile response (1.0 °C increase) to infection compared with healthy, non-challenged controls (Fig. 1). The febrile response peaked 24 h following challenge. As expected, the homologous CA/09 vaccine (Vaccine E) provided significant ($P < 0.0001$) protection against a febrile response following challenge with the same virus. Moreover, the homologous CA/09 vaccine (Vaccine E) was significantly ($P < 0.0001$) better than any of the 3 commercial vaccines (licensed vaccine A and B or autogenous vaccine D) at reducing a febrile response to the virus challenge. All pigs vaccinated with commercial vaccine exhibited significantly ($P < 0.0015$) elevated rectal temperatures versus non-challenged control pigs 24 h following challenge. Although there was no significant difference in protection against the febrile response between any of the 3 vaccines tested (licensed or autogenous), the autogenous vaccine had a significantly reduced febrile response compared with the non-vaccinated, challenged controls ($P = 0.006$) (Fig. 1).

Table 2
Macroscopic pneumonia and microscopic pneumonia lesion severity at 5 days post-infection.[a].

Group	Macroscopic	Microscopic
Vaccine A	3.6 ± 0.6*	0.7 ± 0.4*
Vaccine B	3.5 ± 0.8*	1.4 ± 0.3
Vaccine D	1.8 ± 0.8*	0.4 ± 0.3*
Vaccine E	0.2 ± 0.1*	0.0 ± 0.0*
NV/Challenge	8.2 ± 1.3	2.4 ± 0.2
NV/NC	0.1 ± 0.1*	0.0 ± 0.0*

Vaccine A, fully licensed trivalent vaccine; Vaccine B, fully licensed pentavalent vaccine; Vaccine D, bivalent autogenous vaccine; Vaccine E, monovalent experimental CA/09 vaccine.

[a] NV/Challenge, non-vaccinated, challenged positive control group; NV/NC, non-vaccinated, non-challenged negative control group.

* Significantly different from NV/Challenge control group at $P < 0.05$.

Please cite this article in press as: Vincent AL, et al. Efficacy of inactivated swine influenza virus vaccines against the 2009 A/H1N1 influenza virus in pigs. Vaccine (2010), doi:10.1016/j.vaccine.2010.01.049

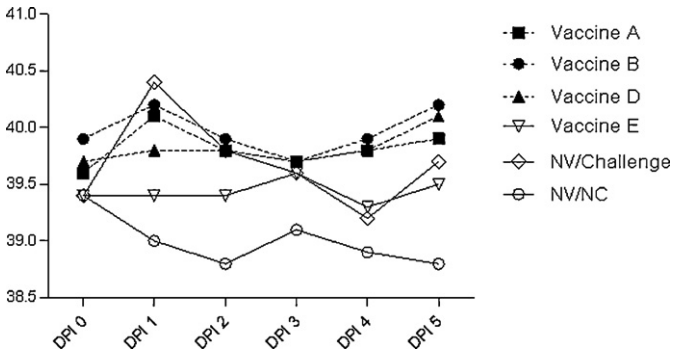

Fig. 1. Rectal temperatures were taken daily from 0 to 5 days post-infection (dpi). The monovalent homologous CA/09 vaccine (Vaccine E) significantly reduced fever compared to the non-vaccinated challenge positive control group. Vaccine A, fully licensed trivalent vaccine; Vaccine B, fully licensed pentavalent vaccine; Vaccine D, bivalent autogenous vaccine; Vaccine E, monovalent experimental CA/09 vaccine; NV/Challenge, non-vaccinated, challenged positive control group; and NV/NC, non-vaccinated, non-challenged negative control group.

3.2. Humoral immune response to vaccines

Homologous vaccine administration induced robust serum HI antibody titers against CA/09, but mean titers in the other vaccine groups were below the detectable threshold or positive cut-off (Table 3). Similar titers were induced against a second 2009 A/H1N1 isolate, A/Mexico/4108/2009 (data not shown). In contrast to the CA/09 HI, the A and D vaccine groups, as well as the homologous vaccine group, all had significant titers to the γ-cluster OH/07 and α-cluster MN/99 swine influenza viruses. Surprisingly, the homologous monovalent CA/09 vaccine group had significant cross-reacting antibody titers to the β-cluster SIV NE/08 as well. The D bivalent vaccine containing both γ- and β-cluster SIV also demonstrated significant HI titers to the β-cluster NE/08 virus as expected.

By ELISA assay, only the D and E groups had levels of IgG significantly higher than negative controls against the 2009 A/H1N1 virus in the serum or BALF (Table 3). The D and E vaccinated pigs were significantly higher in total IgG, but not significantly different from each other. IgA levels were not significantly different than negative controls for any of the inactivated vaccine groups in serum or BALF (data not shown).

4. Discussion

Although the pandemic 2009 A/H1N1 viruses are genetically related to SIV of North American and Eurasian lineages, the constellation of the 8 gene segments in the pandemic A/H1N1 was unique and not known to circulate in pigs prior to the pandemic. Reassortants between the North American and Eurasian lineage swine viruses have been identified from pigs in China [6], but a direct ancestor of the 2009 human pandemic virus has yet to be reported. Additionally, the 2009 pandemic A/H1N1 viruses circulating in the human population have hemagglutinin genes that are phylogenetically distinct from the nearest swine virus sequences, the North American-lineage γ-cluster H1. We have demonstrated that weaned pigs are susceptible to infection and clinical disease induced by CA/09 [11], and others have demonstrated infection of pigs with other isolates of 2009 A/H1N1 [20,21]. The experimental infections in pigs were characteristic of acute influenza illness in swine. Clinical signs, pathologic changes, and virus replication were restricted to the respiratory tract. Likewise, reports of naturally occurring transmission events to pigs suggest that clinical disease is relatively mild and indistinguishable from SIV.

Limited serologic cross-reactivity with the 2009 pandemic A/H1N1 isolates was demonstrated in HI assays with sera from pigs infected or vaccinated with contemporary H1 SIV [11]. Although variation in cross-reactivity between viral antigens was apparent, consistent cross-reactivity was demonstrated between 2009 A/H1N1 and sera from pigs immunized with SIV from the H1γ HA phylogenetic cluster. The cross-reactivity with antisera from these viruses is important since this is the HA cluster to which the HA from the 2009 pandemic H1N1 is most closely related [5]. Thus, pre-existing immunity to certain currently circulating H1 SIV strains may provide some level of protection in pigs against the pandemic virus. Serologic cross-reactivity with antisera from pigs vaccinated with 5 U.S. commercial vaccines was additionally assessed by HI with 3 pandemic H1N1 isolates [11]. Cross-reactivity was consistently low between the vaccine antisera and all 2009 A/H1N1 novel viruses tested, but 3 vaccines (Vaccines A, B, and D) demonstrated some cross-reactivity, suggesting that currently available vaccines may provide some level of protection against infection with the novel human A/H1N1. Here, we investigated a subset of the vaccines from the previous *in vitro* study in a vaccine efficacy experiment to validate the serum cross-reactivity.

In this study, the homologous monovalent inactivated vaccine provided optimal protection against 2009 A/H1N1 challenge in all parameters evaluated. No virus was isolated from the lung or nose at any time points evaluated. Rectal temperatures were significantly reduced over the non-vaccinated challenged controls during the acute 5-day post-infection period, including protection from the peak febrile response at 24 h post-infection. The homologous vaccine also conferred complete protection from macroscopic and microscopic pneumonia. The *in vivo* protection corresponded with

Table 3
Geometric mean reciprocal titers of 0 dpi serum against H1 influenza viruses in the hemagglutination inhibition assay or 0 days post-infection for serum and 5 days post-infection for BALF in a total IgG ELISA.[a].

Group	Hemagglutination inhibition				IgG ELISA	
	MN/99 (α)	NE/08 (β)	OH/07 (γ)	CA/09 pH1N1	CA/09 X-179A	
					Serum	BALF
Vaccine A	92*	17	53*	≤10	≤1000	≤4
Vaccine B	21	11	20	≤10	≤1000	≤4
Vaccine D	92*	53*	113*	12	7131*	8*
Vaccine E	485*	86*	557*	243*	15287*	11.3*
NV/Challenge	≤10	≤10	≤10	≤10	≤1000	≤4
NV/NC	≤10	≤10	≤10	≤10	≤1000	≤4

Vaccine A, fully licensed trivalent vaccine; Vaccine B, fully licensed pentavalent vaccine; Vaccine D, bivalent autogenous vaccine; Vaccine E, monovalent experimental CA/09 vaccine.

[a] NV/Challenge, non-vaccinated, challenged positive control group; NV/NC, non-vaccinated, non-challenged negative control group; BALF, bronchoalveolar lavage fluid. A representative virus from each H1 cluster of North American SIV (α, β, and γ) was included in HI assays.

* Significantly different than NV/NC negative controls.

Please cite this article in press as: Vincent AL, et al. Efficacy of inactivated swine influenza virus vaccines against the 2009 A/H1N1 influenza virus in pigs. Vaccine (2010), doi:10.1016/j.vaccine.2010.01.049

robust HI antibody titers specific to 2009 A/H1N1, only detected in the Vaccine E group. The CA/09 homologous vaccine also induced cross-reacting HI antibodies to the γ-cluster SIV OH/07, but at a higher magnitude than expected. Surprisingly, the CA/09 vaccine antisera also demonstrated significant titers of cross-reacting antibodies to the α-cluster MN/99 and β-cluster NE/08. The Vaccine A commercial trivalent vaccine and the autogenous bivalent Vaccine D induced HI titers to the α-cluster H1 SIV, but significantly lower than the homologous monovalent vaccine. The autogenous bivalent vaccine induced equivalent levels of β-cluster HI antibodies as the CA/09 vaccine, but this was to be expected since the bivalent vaccine included both a β-cluster and a γ-cluster SIV. The lack of response to the β-cluster SIV by Vaccine A antisera and to each of the SIV tested by Vaccine B antisera may indicate a lack of cross-reactivity induced by the vaccine strain to the selected HI test strains or a suboptimal immune response induced by the vaccine. Vaccine B was in the process of being licensed at the time of this experiment, but not yet commercially available, and the vaccine available for testing was at a minimum protective dose (MPD). Commercial SIV manufacturers normally formulate commercial serials of their vaccines at higher HA levels than the MPD and was since done for this vaccine to induce higher HI titers.

The H1 SIV-specific antibodies induced by the commercial and autogenous vaccines are likely to have contributed to the partial protection demonstrated against CA/09 pandemic A/H1N1. Only the homologous vaccine and the autogenous SIV vaccine had significant levels of 2009 A/H1N1-specific IgG in the serum and lower respiratory mucosa measured by ELISA. As the Vaccine D vaccinated pigs had minimal HI activity against the 2009 A/H1N1 isolates tested but had significant protection against CA/09 challenge, it is likely that these non-HI antibodies aided in protection, as was previously shown between subtypes in a mouse model [22]. Of note, all of the vaccines tested provided protection from pneumonia. Importantly, none of the vaccines demonstrated aggravated pneumonia, a phenomenon periodically seen with selected mismatched inactivated vaccine and challenge virus in experimental studies [19,23]. Nonetheless, based on the results demonstrated here, consideration should be given for development of monovalent homologous vaccines to best protect the swine population and to limit shedding of virus, thus reducing the potential transmission of 2009 A/H1N1 among pigs or from pigs to people. As vaccine formulation, including type and amount of adjuvant, is proprietary, the role of adjuvants in inducing the variable responses to the vaccines tested here cannot be ruled out.

Vaccinating pigs against influenza virus is a common practice in the U.S. swine industry. Inactivated influenza vaccines became commercially available in 1994. In 1995, influenza vaccine usage was not reported in the National Animal Health Monitoring System survey of U.S. swine operations [24]. However, by 2000, over 40% of large producers reported that they vaccinated breeding females and approximately 20% vaccinated weaned pigs [25]. In the survey conducted in 2006, the number of producers vaccinating large herds of breeding females increased to 70%, whereas vaccinating weaned pigs remained relatively unchanged [26]. Importantly, of those farms that vaccinated breeding females in 2006, approximately 20% reported using autogenous SIV vaccines rather than commercial vaccines. Autogenous vaccine usage against influenza virus has continued to increase due to the diversity of viruses circulating in the North American pig population and the inability of the animal biologics industry to change the vaccine composition as rapidly as the viruses are changing. In fact, over half of the doses of SIV vaccine released in 2008 were autogenous (Rick Hill, USDA–APHIS–Center for Veterinary Biologics, personal communication). The inclusion of the autogenous vaccine in this study was to understand the current immune status of the U.S. swine population due to prior vaccine usage and not to promote use of γ-cluster autogenous vaccines to protect against 2009 A/H1N1 in U.S. hogs.

Autogenous vaccines have the advantage that they can be produced relatively quickly for use in the herd of origin in response to a specific or unique veterinary need. The disadvantages are that they are only minimally tested for purity and safety, untested for potency and efficacy, and thus cannot be distributed outside the herd of origin. Very limited peer-reviewed scientific information is publicly available on the impact of autogenous vaccine usage in controlling SIV. Fully licensed commercial SIV vaccines must meet the USDA–Center for Veterinary Biologics' requirements for purity, safety, potency, and efficacy, requiring significant investments of time and resources to develop a new product. One of the highest priorities for the recently launched USDA SIV surveillance plan is to enhance monitoring of the evolution of viruses for relevant commercial vaccine strain selection and diagnostic reagent development.

The pig has been suggested to be a "mixing vessel" capable of generating reassorted influenza viruses with pandemic potential due to the presence of both avian and mammalian receptors expressed by respiratory tract epithelial cells [27]. The 2009 pandemic A/H1N1 underscores the potential risk to the human population of other influenza virus subtypes and genotypes with the SIV TRIG backbone and demonstrates the potential for viruses with genes from swine lineages to emerge and cause a pandemic in the human population. Based on the results demonstrated here, consideration should be given for development of monovalent homologous vaccines to be used in all age groups of naive pigs to best protect the swine population and to limit the potential transmission of 2009 A/H1N1 among pigs or from pigs to people. Increased surveillance and monitoring for the pandemic A/H1N1 as well as other SIV in the swine population worldwide are critical to understand the dynamic ecology of influenza A viruses in this susceptible host population. Likewise, development of a vaccine strain selection system through the USDA surveillance plan is critical for controlling SIV and reducing the risk of such reassortment events with the current 2009 A/H1N1 or other emerging viruses in the future.

Acknowledgements

The authors thank Michelle Harland and Hillary Horst for technical assistance and Brian Pottebaum, Jason Huegel, and Dr. Becky Jepsen for assistance with animal studies. Assistance with microbiologic screening from Dr. Susan Brockmeier is appreciated. We thank Dr. Alexander Klimov and Ruben Donis (CDC) for providing pandemic H1N1 isolates. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Funding was provided by USDA-ARS.

References

[1] Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses a North American perspective. Adv Virus Res 2008;72:127–54.
[2] Karasin AI, Carman S, Olsen CW. Identification of human H1N2 and human–swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003–2005). J Clin Microbiol 2006;44(March (3)):1123–6.
[3] Vincent AL, Ma W, Lager KM, Gramer MR, Richt JA, Janke BH. Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. Virus Genes 2009;39:176–85.
[4] Vincent AL, Swenson SL, Lager KM, Gauger PC, Loiacono C, Zhang Y. Characterization of an influenza A virus isolated from pigs during an outbreak of respiratory disease in swine and people during a county fair in the United States. Vet Microbiol 2009;137:51–9.
[5] Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009;325(July (5937)):197–201.

[6] Smith GJD, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009;459(June):1122–5.
[7] Trifonov V, Khiabanian H, Rabadan R. Geographic dependence, surveillance, and origins of the 2009 influenza A (H1N1) virus. N Engl J Med 2009;361(May (2)):115–9.
[8] Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. Clin Infect Dis 2007;44(April (8)):1084–8.
[9] Shinde V, Bridges CB, Uyeki TM, Shu B, Balish A, Xu X, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. N Engl J Med 2009;360(May (25)):2616–25.
[10] USDA–Animal and Plant Health Inspection Services VS. Code of Federal Regulations, Title 9 Animals and Animal Products. Washington, DC [updated December 30, 2009; cited 2010 January 18]. Available from: http://edocket.access.gpo.gov/cfr_2009/janqtr/pdf/9cfr113.113.pdf.
[11] Vincent AL, Lager KM, Faaberg KS, Harland M, Zanella EL, Ciacci-Zanella JR, et al. Experimental inoculation of pigs with pandemic H1N1 2009 and HI cross-reactivity with contemporary swine influenza virus antisera. Influenza Other Respir Viruses; in press.
[12] Halbur PG, Paul PS, Frey ML, Landgraf J, Eernisse K, Meng XJ, et al. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet Pathol 1995;32(6):648–60.
[13] Richt JA, Lager KM, Janke BH, Woods RD, Webster RG, Webby RJ. Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses cocirculating in the United States. J Clin Microbiol 2003;41(July (7)):3198–205.
[14] Opriessnig T, Yu S, Gallup JM, Evans RB, Fenaux M, Pallares F, et al. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet Pathol 2003;40(September (5)):521–9.
[15] Strait EL, Madsen ML, Minion FC, Christopher-Hennings J, Dammen M, Jones KR, et al. Real-time PCR assays to address genetic diversity among strains of *Mycoplasma hyopneumoniae*. J Clin Microbiol 2008;46(August (8)):2491–8.
[16] Kitikoon P, Nilubol D, Erickson BJ, Janke BH, Hoover T, Sornsen S, et al. The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination. Vet Immunol Immunopathol 2006;112(August (3–4)):117–28.
[17] Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938;27:493–7.
[18] WHO manual on animal influenza diagnosis and surveillance; 2002 [cited 2009 May 25]. 2nd:[Available from: http://www.wpro.who.int/NR/rdonlyres/EFD2B9A7-2265-4AD0-BC98-97937B4FA83C/0/manualonanimalaidiagnosisandsurveillance.pdf.
[19] Vincent AL, Lager KM, Janke BH, Gramer MR, Richt JA. Failure of protection and enhanced pneumonia with a US H1N2 swine influenza virus in pigs vaccinated with an inactivated classical swine H1N1 vaccine. Vet Microbiol 2008;126(January (4)):310–23.
[20] Lange E, Kalthoff D, Blohm U, Teifke JP, Breithaupt A, Maresch C, et al. Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs. J Gen Virol 2009;90(September (Pt 9)):2119–23.
[21] Brookes SM, Irvine RM, Nunez A, Clifford D, Essen S, Brown IH, et al. Influenza A (H1N1) infection in pigs. Vet Rec 2009;164(June (24)):760–1.
[22] Sambhara S, Kurichh A, Miranda R, Tumpey T, Rowe T, Renshaw M, et al. Heterosubtypic immunity against human influenza A viruses, including recently emerged avian H5 and H9 viruses, induced by FLU-ISCOM vaccine in mice requires both cytotoxic T-lymphocyte and macrophage function. Cell Immunol 2001;211(August (2)):143–53.
[23] Kitikoon P, Vincent AL, Janke BH, Erickson BJ, Strait EL, Yu S, et al. Swine influenza matrix 2 (M2) protein contributes to protection against infection with different H1 swine influenza virus (SIV) isolates. Vaccine 2009;38(December (2)):523–31.
[24] USDA. In: USDA:APHIS:VS C, editor. Trends in vaccination practices on U.S. swine operations. Fort Collins, CO: USDA; 1995.
[25] USDA. In: USDA:APHIS:VS C, editor. Trends in vaccination practices for respiratory diseases on U.S. swine operations – 2000. Fort Collins, CO: USDA; 2003.
[26] USDA. In: USDA:APHIS:VS C, editor. Swine 2006. Part II. Reference of swine health and health management practices in the United States, 2006. Fort Collins, CO: USDA; 2007.
[27] Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 1998;72(September (9)):7367–73.

Please cite this article in press as: Vincent AL, et al. Efficacy of inactivated swine influenza virus vaccines against the 2009 A/H1N1 influenza virus in pigs. Vaccine (2010), doi:10.1016/j.vaccine.2010.01.049