Sponsors

University of Minnesota
College of Veterinary Medicine

College of Agricultural, Food and Environmental Sciences

Extension Service

Swine Center

Production Assistants
Steven Claas
Lynn Leary

Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Evaluation of the ability of vaccines to cross-protect against a serologically and genetically diverse H3N2 virus variant

JeeHoon Lee, Marie Gramer, HanSoo Joo
Swine Group, University of Minnesota

Introduction

Three different subtypes of swine influenza virus (SIV) have been identified in U.S. swine. Before 1998, only classical H1N1 virus was prevalent in the swine population. H3N2 viruses with genes derived from human, swine and avian influenza viruses have been evident since 1999. Shortly after that, H1N2 virus, a result of the reassortment produced between the triple reassortant H3N2 and classical H1N1 viruses, has also been isolated from pigs (Karasin et al 2000; Choi et al 2002).

Sequence analysis of the triple reassortant H3N2 viruses has shown that their HA genes belong to one of the three phylogenetically distinct HA lineages. Therefore, H3N2 viruses have been classified into three distinct genetic clusters (Webby et al., 2000; Richt et al., 2003). At present, however, it is not known whether the H3N2 viruses in the different clusters are antigenically distinct. Reports from veterinary diagnostic laboratories showed increasing cases of H3N2 isolates with low HI antibody titers to the reference H3N2 virus antiserum. In addition, vaccination failures with commercial SIV vaccines have been reported from swine farms.

It could be possible that immunity induced by current commercial bivalent SIV vaccines is not satisfactory to provide sufficient protection against the H3N2 variants. Therefore, the objective of this study is to investigate whether the 3 USDA licensed bivalent vaccines could provide satisfactory protection against a H3N2 serologic variant.

Materials and methods

Experimental design

The experimental design is outlined in **Table 1**. Forty-four 3-week old pigs were randomly divided into 6 groups. All pigs were free of PRRS virus, *M. hyopneumoniae* and SIV infections. The pigs in groups 1, 2, and 3 were vaccinated twice with commercial bivalent SIV vaccines X, Y, and Z, respectively. An experimental homologous H3N2 virus vaccine was prepared using the H3N2 challenge virus and injected twice intramuscularly to pigs in group 4. Pigs in groups 5 and 6 (4 pigs) served as controls without vaccination. Two weeks after the second vaccination, all pigs were inoculated intranasally with the challenge H3N2 virus (A/Sw/CO/00294/2004) at $10^{8.5}$ $TCID_{50}$/ml.

Challenge virus and SIV vaccines

The selection of SIV H3N2 virus (A/Sw/CO/00294/2004) as a challenge virus was based on the phylogenetic and serologic analyses. The virus was isolated from a 10-week old pig in a farm with a history of severe influenza induced clinical signs and possible vaccination failure. Three USDA licensed SIV bivalent vaccines X, Y, and X were purchased from a commercial source. An inactivated vaccine was prepared using the challenge virus. The virus was grown in MDCK cells (HA titer 1:256/0.1 ml), inactivated with formalin and mixed with a mineral oil adjuvant. All of the vaccines were stored at -4C until use.

Clinical observation, sampling, gross examination, and serology

Clinical signs were monitored every morning for 30 min. Daily rectal temperature and nasal swabs were collected. Blood samples were collected from all pigs at intervals.

Table 1 Experimental design.

Group	Vaccination 0 & 2 weeks	Challenge 4 weeks	Necropsy Day post challenge
1 (*n* = 8)	Vaccine X	Yes	5 and 6
2 (*n* = 8)	Vaccine Y	Yes	5 and 6
3 (*n* = 8)	Vaccine Z	Yes	5 and 6
4 (*n* = 8)	Homologous vaccine	Yes	5 and 6
5 (*n* = 8)	Challenge only	Yes	5 and 6
6 (*n* = 4)	No vaccine & challenge	No	5 and 6

At 5 or 6 days post-challenge (dpc), pigs were euthanized, and their lungs were examined grossly and histologically. Each lung was given blindly to one examiner to avoid bias, and percentages of lung consolidation were scored (mild lesions - <10%; moderate lesion - 10-20%; severe lesion - > 20%). Nasal swabs were examined for the presence of SIV using MDCK cells by a routine method. All sera were tested for SIV antibody by HI test (Direksin et al., 2002). The HI tests were performed against both challenge virus (A/Sw/CO/00294/2004) and reference H3N2 virus (A/SW/TX/4199-2/98). The HI titers of <1:40 were considered as negative.

Results

Serologic and genetic characteristics of the challenge H3N2 virus

To examine the serologic cross reactivity among H3N2 viruses, antisera were prepared by collecting sera from pigs inoculated with 5 selected H3N2 viruses - A/SW/NC/39615/01 (A), A/SW/MO/22582/02 (B), A/SW/NC/5854/02 (C), A/SW/MN/23062/02 (D), and A/SW/MN/46710-35 (E). The viruses were selected based on their genetic relationships in a phylogenetic tree. Antisera were also prepared by collecting sera from pigs following inoculations with SIV commercial vaccine X, Y or Z. Using the challenge virus (A/Sw/CO/00294/2004) in the HI test, HI titers were low (<1:80) with antisera to commercial SIV vaccines but high (1:1,280) with antisera to virus C and D (**Table 2**). The challenge virus was classified as a cluster III virus according to the HA gene nucleotide sequence analysis.

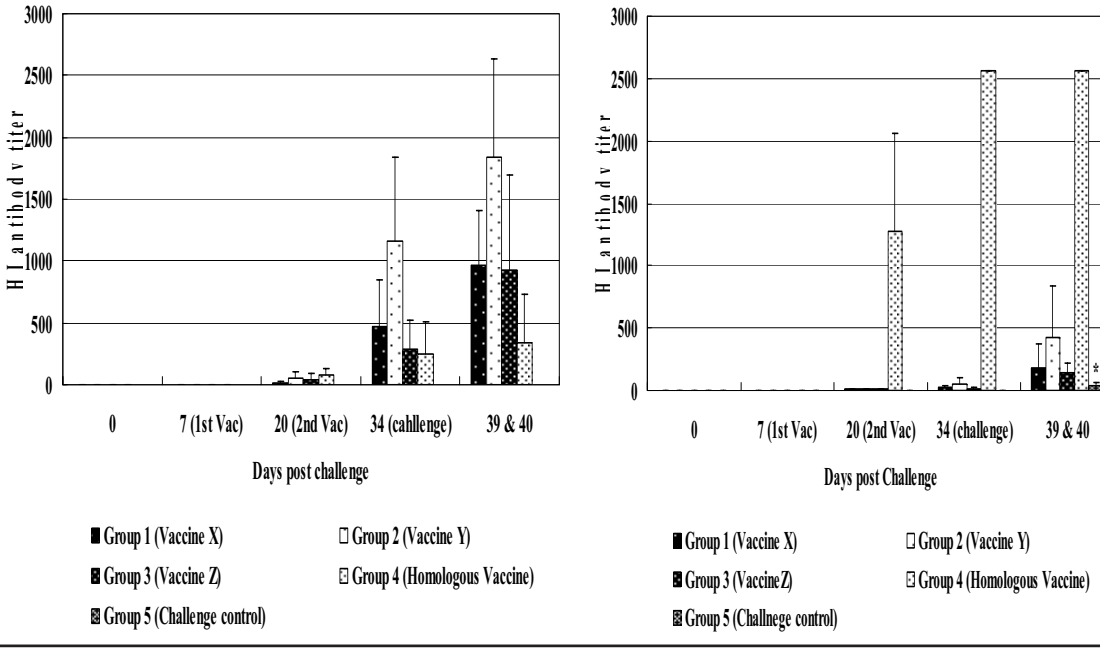
Antibody response to vaccination

The HI antibody titers against a reference H3N2 virus (A/SW/TX/4199-2/98; cluster I) and the challenge virus (A/Sw/CO/00294/2004 – cluster III) in pigs following inoculation with different vaccines and challenge are summarized in **Figure 1**. A marked difference in HI titers was observed when the sera were tested using the 2 different H3N2 viruses. Using the reference H3N2 virus, low HI antibody titers were detected at 14 days after the first vac-

Table 2: HI titers of pig sera immunized with different H3N2 isolates or commercial SIV vaccines against the challenge virus (A/Sw/CO/00294/2004) and a reference virus (H3N2 A/Sw/TX/4199-2/98).

Triple reassortant H3N2 is0lates	Phylogenetic cluster	Antiserum against H3N2 virus or commercial vaccine							
		A	B	C	D	E	X	Y	Z
A/Sw/CO/00294/2004	III	-	40	1280	1280	20	80	20	40
A/Sw/TX/4199-2/98	I	20	40	320	160	40	640	640	640

Figure 1: Mean HI antibody titers in pigs following inoculation of different SIV vaccines. The HI test was performed using a reference H3N2 virus (A/Sw/TX/4199-2/98–left graph) or challenge virus (A/Sw/CO/00294/2004–right graph).



cination but the titers increased to 1:80-2,560 at 2 weeks after the second vaccination with 3 commercial vaccines (groups 1, 2 and 3). The HI antibody titer curve was similar in pigs inoculated with the homologous vaccine (group 4). Conversely, using A/Sw/CO/00294/2004 H3N2 virus, HI antibody titers in pigs with 3 commercial vaccines were not detected after the first vaccination and were still negative or low at 14 days after the second vaccination. However, pigs inoculated with the homologous vaccine (group 4) showed a very high antibody response (1:640-2,560) even at 14 days after the first vaccination. At the time of euthanasia, all vaccinated pigs in groups 1-4 had positive antibodies against the challenge virus. Pigs in group 5 showed positive HI titers against the challenge virus in 3 of the 8 pigs after 5-6 dpc. All pigs in group 6 remained negative throughout the experimental period.

Clinical signs and virus shedding following challenge

On 1 and 2 dpc, some pigs showed high fever over 40_ but there was no significant difference in the temperatures between the groups. Clinical signs in the challenge only group were typical to that of SIV infection, while pigs in group 4 showed markedly reduced clinical signs. Clinical signs in pigs given commercial vaccines were milder than non-vaccinated, challenged pigs. Four control pigs without challenge had no clinical signs during the experiment. Virus was isolated from nasal swabs of all pigs in group 5 between 1 and 4 dpc, while no virus was isolated from pigs vaccinated with the homologous vaccine (group 4). Virus was also isolated from nasal swabs of most pigs in groups 1, 2, and 3 between 1 and 4 dpc but viral shedding was reduced on day 5 in groups 1, 2, and 3 compared to non-vaccinated, challenged controls (group 5).

Gross lung lesions

Mean lung lesion score for non-vaccinated, challenged pigs (group 5) was 16.92 ± 10.30% and significantly higher ($P < 0.0001$) than those of the vaccinated groups 1, 2, 3 and 4. Three of 8 pigs in groups 2, 3 and 4 had average lung scores of 0.73 ± 1.74%, 1.72 ± 2.51%, and 0.28 ± 0.44% respectively, but the groups' mean lung lesion scores were not statistically significant. Six of 8 pigs in group 1 had mean scores of 3.54 ± 3.57%, and the score was statistically higher ($P < 0.05$) compared to the scores of the homologous vaccinated group (group 4).

Microscopic lung lesions

The nasal turbinates and trachea were examined for epithelial changes and subepithelial inflammation. The lungs were examined for bronchiolar epithelial changes. Non-vaccinated, challenged pigs (group 5) had mild to moderate bronchiolar epithelial damage and while all vaccinated pigs (groups 1, 2, 3 and 4) had minimal to no bronchiolar epithelial damage. Nasal turbinate and tracheal changes in all groups were minimal to mild.

Discussion

Control of SIV using a vaccine has become a routine method in swine farms. However, it is often complicated because of the prevalence of different antigenic subtypes. Several cross-protection studies with different SIV vaccines have been performed in Europe and the U.S. (Van Reeth et al., 2001, 2003; Heinen et al., 2001; Gramer and Rossow, 2004), and the results showed that the vaccines were efficacious under experimental conditions (Gramer and Rossow, 2004). Failure of successful protection with commercial SIV vaccines was also reported under field condition due to infections with viruses of antigenic and genetic diversity (Van Reeth et al., 2001; Webby et al., 2004).

In this study, the challenge virus with highest diversity by both genetic and serologic analyses was selected. The challenge virus was classified in genetic cluster III, while the commercial vaccines contained H3N2 viruses in genetic cluster I. The challenge virus demonstrated low serologic cross-reaction to the antisera induced by current commercial vaccines. The 3 commercial vaccines were effective in reducing clinical signs and lung lesion scores upon challenge. However, shedding of the challenge virus was observed in pigs given commercial vaccines. While duration of viral shedding in the commercially vaccinated groups was similar to the unvaccinated group, less vaccinated pigs were shedding virus at 5 dpc. In contrast, virus shedding was not observed in the pigs of homologous vaccine group. The unsuccessful prevention of challenge virus shedding is concerning. If the vaccines are only able to partially reduce virus shedding, the virus may become more diverse and possibly pose a risk to other animals or humans (Heinen et al., 2001; Ritch et al., 2003).

In summary, some of the SIV vaccines used in the U.S. today are bivalent vaccines containing both H3N2 (cluster I) and H1N1 subtypes and claims. Consistent with their label claims, all the current commercial vaccines reduced clinical signs and reduced lung lesion but did not prevent virus infection and shedding. On the other hand, more complete protection was observed with the homologous virus vaccine. It is interesting to note that lung lesions were significantly higher in one of the commercial vaccinated groups in comparison to the homologous vaccinated group. It may be necessary to review and update the SIV strains in each vaccine with continuous monitoring of the field isolates for their genetic and serologic diversity.

Acknowledgements

This work has been funded by Pork Checkoff, Minnesota Pork Board.

References

Choi YK, Goyal SM, and Joo HS (2002) Prevalence of swine influenza virus subtypes on swine farms in the United States. *Arch Virol* 147: 1209-1220.

Direksin K, Joo HS, and Goyal SM (2002) An immunoperoxidase monolayer assay for the detection of antibodies against swine influenza virus. *J Vet Diagn Invest* 14:169-171.

Gramer M. and Rossow K (2004) Epidemiology of swine influenza and implications of reassortment. *Allen D. Leman Swine Conference*, p. 69-73

Heinen PP, van Nieuwstadt AP, de Boer-Luijtze EA, and Bianchi AT (2001) Analysis of the quality of protection induced by a porcine influenza A vaccine to challenge with an H3N2 virus. *Vet Immunol Immunopathol* 28:39-56.

Karasin AI, Olsen CW and Anderson GA (2000) Genetic characterization of an H1N2 influenza virus isolated from a pig in Indiana. *J Clin Microbiol* 38: 2453-2456.

Richt JA, Lager KM, Janke BH, Woods RD, Webster RG, and Webby RJ (2003) Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses co-circulating in the United States. *J Clin Microbiol* 41:3198-3205.

Van Reeth K, Labarque G, De Clercq S, and Pensaert M (2001) Efficacy of vaccination of pigs with different H1N1 swine influenza viruses using a recent challenge strain and different parameters of protection. *Vaccine* 19:4479-4486.

Van Reeth K, Gregory V, Hay A, and Pensaert M (2003) Protection against a European H1N2 swine influenza virus in pigs previously infected with H1N1 and/or H3N2 subtypes. *Vaccine* 21:1375-1381.

Webby RJ, Swenson SL, Krauss SL, Gerrish PJ, Goyal SM, and Webster RG (2000) Evolution of swine H3N2 influenza viruses in the United States. *J Virol* 74: 8243-8251.

Webby RJ, Rossow K, Erickson G, Sims Y, and Webster R (2004) Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population. *Virus Res* 103:67-73.