FluSure XP™ Update with Regards to 2009 H1N1 “Swine Influenza” Virus

Although the 2009 H1N1 “swine influenza” virus has not been identified in US swine herds, it should be noted that swine influenza vaccines, including FluSure XP likely may offer no or very limited protection against the recently identified H1N1 influenza virus, often referred to as “Swine Flu”. Pfizer Animal Health is working with research and regulatory agencies around the world to address this emerging situation, including the rapid development of a vaccine solution.
Efficacy of FluSure XP™ in pigs challenged with either a heterologous reassortant H3N2 or H1N1 swine influenza virus

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Key Points

• Two studies were conducted to evaluate the efficacy of trivalent FluSure XP following challenge with either a Cluster IV H3N2 or a human-like H1N1 swine influenza virus (SIV).1,2

• When compared with pigs in the placebo control group (controls), pigs in the group vaccinated with FluSure XP (vaccinates) and challenged with a H3N2 virus had significantly less virus isolated from nasal swabs on post-challenge days 2, 3, and 4 (P ≤ 0.0005 each day) and from bronchoalveolar lavage (BAL) fluids at necropsy (P ≤ 0.0001).1

• Vaccinates challenged with a H1N1 virus had significantly less virus isolated from nasal swabs on post-challenge days 3, 4, and 5 (P ≤ 0.0001 each day) and from BAL fluids at necropsy (P ≤ 0.0005) when compared with controls.2

• Vaccinates challenged with a Cluster IV H3N2 virus had significantly lower least squares (LS) mean lung lesion scores at necropsy (0.4% vs 3.9%; P ≤ 0.0001) when compared to controls.1

• Vaccinates challenged with a human-like H1N1 virus had numerically lower LS mean lesion scores at necropsy (2.6% vs 4.0%; P > 0.05) when compared to controls.2

• Analysis by stratified mitigated fraction showed that vaccination with FluSure XP mitigated the effects of both H3N2 and H1N1 induced lung lesions.1,2

• Virus shedding in nasal swabs was also significantly lower in vaccinates in both studies by area under the curve (AUC) analysis for all post-challenge days (P ≤ 0.0001) and by percent age of pigs ever positive (P ≤ 0.05 in the H3N2 study; P ≤ 0.0001 in the H1N1 study).1,2

• Vaccinates had significantly (P ≤ 0.0001) higher hemagglutination inhibition (HI) antibody titers to the H3N2 and H1N1 vaccine viruses and challenge viruses than controls.1,2
FluSure XP from Pfizer Animal Health is the first swine influenza vaccine licensed under the new United States Department of Agriculture (USDA) recommendations that are contained in Veterinary Services (VS) Memorandum No. 800.111 (September 2007). VS Memorandum No. 800.111 helps accelerate the updating of strains in currently licensed inactivated swine and equine influenza vaccines. The human-like H1N1, legacy FluSure H1N2-like, and Cluster IV H3N2 vaccine strains selected for the new FluSure XP formulation were chosen because they were genetically similar to and broadly cross-reactive with a panel of contemporary field isolates representing the major genetic clusters currently circulating in US swine herds. To comply with the new USDA recommendations, Pfizer Animal Health was required to demonstrate immunogenicity in host animals by serology; that is, pigs vaccinated with the newly updated strains in FluSure XP vaccine needed to generate immune responses that were not less than those produced by the legacy FluSure formulation. Additional challenge studies and field safety studies were not required.

In the pivotal immunogenicity study, pigs vaccinated with the new trivalent FluSure XP vaccine responded with HI antibody titers to the vaccine viruses that were similar to the HI antibody titers of pigs vaccinated with the legacy bivalent FluSure vaccine, thereby fulfilling the requirements of VS Memorandum No. 800.111. In all pigs in all treatment groups, individual HI titers to the vaccine viruses were ≥ 40, the titer considered to be protective against swine influenza.

Based on the results of the immunogenicity study, Pfizer Animal Health received a license to produce and market FluSure XP in the US. Although no more testing was required for licensing the new vaccine, Pfizer Animal Health conducted two additional host animal challenge-of-immunity studies to further assess the efficacy profile of the new human-like H1N1 and Cluster IV H3N2 SIV vaccine strains. These studies are summarized in this bulletin to help practitioners and producers make informed decisions as to whether FluSure XP would be expected to help protect against H1 and H3 viruses from the predominant genetic clusters currently circulating in the US swine population. Previous vaccination and challenge studies conducted with the legacy FluSure vaccine established that the H1N2-like vaccine virus helps protect pigs against disease caused by H1N2-like, reassortant H1N1, and classic H1N1 SIVs.

**Additional FluSure XP Efficacy Studies**

**Similar study designs**

In each study, three-week-old pigs seronegative by HI to H1N1 and H3N2 viruses were randomly allocated to one of four treatment groups (11–15 pigs/group) or to a non-treated (NTX) placebo control group (5 pigs/study). Pigs were vaccinated intramuscularly twice, two weeks apart, with an adjuvanted placebo control (controls) or with three different doses of trivalent FluSure XP. For each study, only data for the controls and the group of pigs vaccinated with the minimum release vaccine dose of FluSure XP (vaccinates) are presented in this bulletin.

Two weeks after the second vaccination, pigs were challenged by endotracheal inoculation with a Cluster IV H3N2 virus (Study 1) or a human-like H1N1 virus (Study 2). The challenge viruses were heterologous to the vaccine strains (Figures 1 and 2). In both studies, clinical observations and nasal swabs for virus isolation were collected prior to challenge and daily after challenge. Blood samples were collected for HI antibody testing prior to the first and second vaccination, prior to challenge, and again five days after challenge. At five days post-challenge, pigs were euthanized and submitted for necropsy examination; lung lesions were scored; and bronchoalveolar lavage (BAL) fluids were collected for virus isolation. The animal phase of each study was conducted according to the guidelines of Pfizer Animal Health’s Institutional Care and Use Committee.
Figure 1—Phylogenetic relationship of H3N2 vaccine and challenge viruses, based on sequencing of the first 900 bases and alignment of ~600 bases of the hemagglutinin genes (Dr. M. Gramer, University of Minnesota Veterinary Diagnostic Laboratory).

Figure 2—Phylogenetic relationship of H1N1 vaccine and challenge viruses, based on sequencing of the first 900 bases and alignment of ~600 bases of the hemagglutinin genes (Dr. M. Gramer, University of Minnesota Veterinary Diagnostic Laboratory).
**Analysis**

The variables analyzed were macroscopic lung lesions (percent involvement), HI antibody titers, virus isolation from nasal swabs and BAL, clinical observations, and rectal temperatures. The 5% level of significance (P ≤ 0.05) was used to assess statistical differences (SAS/STAT Version 9.1). Lung lesions were also evaluated by stratified mitigated fraction (see box below), another statistical method for assessing vaccine efficacy in challenge studies. Additionally, area under the curve (AUC) was used for analysis of virus isolation from nasal swabs. Data from the NTX pigs—euthanized and examined on the day prior to challenge—were not included in the analysis.

**H3N2 Cluster IV challenge study results**

*Percentage of Total Lung with Lesions*

There was no evidence of SIV exposure in the NTX pigs. Compared with controls, vaccinates challenged with an H3N2 Cluster IV virus had significantly lower LS mean lung lesion scores (0.4% versus 3.9%; P ≤ 0.0001) at necropsy examination (Figure 3). Analysis by stratified mitigated fraction demonstrated that vaccination with FluSure XP mitigated the effect of the challenge in producing lung lesions. The lower 95% confidence interval (CI) for vaccinates compared with controls was greater than zero, indicating that FluSure XP reduced the effects of the H3N2 challenge in producing lung lesions.

**Virus Isolation**

Compared with controls, vaccinates challenged with an H3N2 virus had significantly less virus isolated from nasal swabs on post-challenge days 2, 3, and 4 (P ≤ 0.0005 each day) and from BAL fluids at necropsy (P ≤ 0.0001) (Figure 4). Controls were positive for viral shedding as early as one day after challenge, and the percentage of pigs ever positive for virus was significantly lower for the vaccinates than for the controls (36.4% and 91.7%, respectively; P ≤ 0.05). Only one vaccinate was positive for SIV in BAL fluids compared with 11 of 12 positive controls. Virus shedding in nasal swabs was also significantly (P ≤ 0.0001) lower in vaccinates when data were analyzed using AUC over all post-challenge days (data not shown).

**Clinical Signs And Serologic Results**

Clinical signs of respiratory disease in this study were mild and infrequently observed. Compared with controls, vaccinates had significantly higher HI antibody titers (P ≤ 0.0001) to the H3N2 vaccine virus and the challenge virus in sera collected on the day prior to challenge and at necropsy (Figure 5). The higher HI titers at five days post-challenge in vaccinates likely reflected an anamnestic boost to challenge virus and demonstrated cross-reactivity between the H3N2 vaccine virus and the heterologous challenge strain.

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*What Is Stratified Mitigated Fraction?*

Stratified mitigated fraction provides a measure of the relative increase in probability that vaccinates disease will be less severe than non-vaccinates disease. This measure is routinely used in human medicine to quantify the effect of interventions that reduce the severity of a disease outcome without entirely preventing it.
H1N1 challenge study results

Percentage of Total Lung with Lesions

There was no evidence of SIV exposure in the NTX pigs. All of the controls were positive for lung lesions at necropsy examination. In comparison, vaccinates challenged with H1N1 virus had numerically lower LS mean lung lesion scores, but the differences were not significant (P > 0.05) when analyzed by the generalized linear mixed model (Figure 6). Although there were no significant differences in LS mean lung lesion scores, analysis by stratified mitigated fraction demonstrated that vaccination with FluSure XP reduced the effect of the H1N1 challenge virus in producing lung lesions. The lower 95% confidence interval (CI) for the vaccinates was greater than zero, indicating that the minimum release vaccine dose of FluSure XP reduced the effects of the H1N1 challenge in producing lung lesions.

Figure 4—H3N2 virus isolation from nasal swabs and bronchoalveolar lavage fluids (Study 1).

Figure 5—Hemagglutination inhibition antibody titers to H3N2 vaccine and challenge strains (Study 1).
Compared with controls, vaccinates challenged with an H3N2 virus had significantly less virus isolated from nasal swabs on post-challenge days 2, 3, and 4 (P ≤ 0.0005 each day) and from BAL fluids at necropsy (P ≤ 0.0001) (Figure 4). Controls were positive for viral shedding as early as one day after challenge, and the percentage of pigs ever positive for virus was significantly lower for the vaccinates than for the controls (36.4% and 91.7%, respectively; P ≤ 0.05). Only one vaccinate was positive for SIV in BAL fluids compared with 11 of 12 positive controls. Virus shedding in nasal swabs was also significantly (P ≤ 0.0001) lower in vaccinates when data were analyzed using AUC over all post-challenge days (data not shown).

**Clinical Signs And Serologic Results**

Clinical signs of respiratory disease in this study were mild and infrequently observed. Compared with controls, vaccinates had significantly higher HI antibody titers (P ≤ 0.0001) to the H1N1 vaccine and challenge viruses in sera collected on the day prior to challenge and at necropsy (Figure 8). These higher post-challenge titers in the vaccinates most likely reflected a boosted response to the challenge virus and demonstrated cross-reactivity between the human-like H1N1 vaccine and challenge strains.

**Conclusions**

The studies summarized in this bulletin further define the efficacy profile of the new trivalent SIV vaccine FluSure XP. Whereas the pivotal immunogenicity licensing study established that pigs vaccinated with FluSure XP had a similar HI antibody response to pigs vaccinated with the legacy bivalent FluSure, the two additional
challenge studies demonstrated efficacy against experimental challenge of pigs with heterologous viruses belonging to two newly emerging SIV genetic clusters (Cluster IV H3N2 and human-like H1N1). In both studies, when compared to placebo controls, FluSure XP helped:

- Reduce lung lesions caused by challenge virus
- Reduce virus shedding in nasal swabs
- Reduce virus isolations from BAL fluids
- Stimulate significant circulating HI antibody titers

Of practical importance to the hog industry is the fact that vaccination with FluSure XP significantly reduced the incidence and amount of nasal shedding and virus isolated from BAL fluids within days of the SIV challenge. Because vaccination significantly \( (P \leq 0.05) \) decreased the amount of SIV in both the lungs and nasal cavities, it may be effective in altering the disease dynamics within a barn, site, or system by helping decrease the level of virus within a group of pigs, thereby helping reduce the viral exposure to other pigs.

In both studies, vaccination with FluSure XP generated high HI antibody titers to the vaccine and challenge virus strains. Identity testing confirmed that both the heterologous H3N2 and H1N1 challenge viruses used in these studies were representative of major genetic clusters now evident in the US swine population. Serologic and challenge results presented above suggest that FluSure XP has been appropriately updated for meeting the evolutional changes in contemporary H3N2 and H1N1 viruses brought about through antigenic drift and antigenic shift. Previous vaccination and challenge studies conducted with the legacy H1N1 strain in FluSure XP established that the vaccine virus also helps protect pigs against disease caused by H1N2-like, reassortant-like H1N1, and classic H1N1 viruses. Thus, based on the results of the challenge studies reported here as well as HI cross-reactivity, the SIV strains in FluSure XP would be expected to help cross-protect against H1 and H3 SIVs from the predominant genetic clusters currently circulating in the US swine population. Currently, only FluSure XP has been formulated to help provide a broad level of protection based on the use of vaccines with the closest possible match between vaccinal strains and circulating SIV strains.

*Figura 8—Hemagglutination inhibition antibody titers to H1N1 vaccine and challenge strains (Study 2).*
References


