

Technical Update

Updated FluSure XP® Offers Cross-Protection in Pigs Challenged with Contemporary Cluster IV-A H3N2 Swine Influenza Virus^{1,2}

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The changing genetic and antigenic characteristics of swine influenza A virus (IAV-S) field strains continues to menace veterinarians and producers trying to help prevent respiratory disease caused by IAV-S. Because of the rapid pace of IAV-S genetic changes and transmission, vaccines used for disease prevention must occasionally be updated to maintain efficacy against the most important IAV-S subtypes and clusters currently circulating in swine.

FluSure XP® vaccine from Zoetis provides an example of the update process in response to on-going research and pathogen surveillance. FluSure XP was originally licensed in 2008 as a trivalent formulation containing H1N1 Gamma cluster, H1N1 Delta-2 cluster, and H3N2 Cluster IV viruses, and the formulation was subsequently updated in 2011 with the addition of an H1N2 Delta-1 cluster virus to help further the scope of protection. However, new IAV-S field strains capable of imposing serious health threats have emerged since that time, so FluSure XP has again been updated to help ensure optimal protection for US herds.

A novel influenza subtype, pandemic H1N1 (pH1N1), appeared in the US swine popula-

tion in 2009 and rapidly spread both in the US and throughout the world. Since then, evaluations of US field viruses have demonstrated multiple reassortant events between pH1N1 and endemic IAV-S, resulting in H3 and H1 variant viruses with internal genes from pH1N1, most notably the pandemic Matrix ('M') gene of Eurasian swine origin.³⁻⁵ Surveillance data, based on sequencing of the hemagglutinin (HA) gene at the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL), showed that contemporary Cluster IV H3 viruses and Gamma-cluster H1 viruses are genetically diverse, and many of these contemporary viruses contain the pandemic M gene.^{6,7} Additional recent surveillance data has further distinguished H3N2

Subcluster IV-A and IV-B viruses as the most epidemiologically important subclusters circulating in US swine.⁸ Six phylogenetic clades of Cluster IV H3N2 have emerged in US swine (IV-A through IV-F).^{7,9-10} The average pairwise nucleotide distance between clades ranges from 5.4% to 7.6%, and the most prevalent clade is IV-A, representing 76.68% of cataloged H3N2 viruses.¹⁰

To help meet these emerging IAV-S threats, a new update to FluSure XP involved the following antigenic adjustments:

FluSure XP® antigens have been updated to strains genetically closer to those circulating in US herds.

- 2 new H3N2 Cluster IV strains replaced the previous single H3N2 fraction (A/swine/Missouri/069/05) and expanded protection:
 - 1) A/swine/North Carolina/394/2012 ('H3N2 Isolate NC/394'), a Cluster IV-A virus;
 - 2) A/swine/Minnesota/872/2012, a Cluster IV-B virus.

These 2 new H3N2 Cluster IV antigens are closer genetically and antigenically to those currently circulating in the US swine population.^{7,9-12}

- Removal of the A/swine/North Carolina/031/05/H1N1 (Delta 2) virus because surveillance data indicated it is no longer epidemiologically important in the US.^{7,10}

The new FluSure XP formulation helps meet the changing antigenic picture of IAV-S by containing viruses representing H3 Subclusters IV-A and IV-B, the H1 Gamma clade, and the H1 Delta 1 clade. Field surveillance data confirm these viruses as the most prevalent in US swine herds,⁶ thus supporting their selection for use in the new formulation. Of course, clinical research was needed to gain licensure of the updated FluSure XP formulation by the USDA. A summary of some of that research follows, a study that evaluated the cross-protective efficacy of the H3N2 Isolate NC/394 fraction of the new FluSure XP formulation in swine challenged with a contemporary, virulent field isolate of Cluster IV-A H3N2 virus containing the M gene.^{1,2}

FluSure XP®

FluSure XP is a killed vaccine indicated for the vaccination of healthy swine, including pregnant sows and gilts, 3 weeks of age or older as an aid in preventing respiratory disease caused by IAV-S subtypes H1N1, H1N2, and H3N2. A sterile diluent containing Amphigen® is used to aseptically rehydrate the freeze-dried vaccine. Swine should receive two 2-mL doses administered intramuscularly (IM) approximately 3 weeks apart.

Experiment Design

The IAV-S cross-protection study involved 45 weaned piglets (barrows and gilts) approximately 3 weeks of age that were obtained from a high-health-status producer in Minnesota and transported to a Michigan research facility. Piglets had no history of exposure to PRRSV or *Mycoplasma hyopneumoniae* and no exposure or vaccination to IAV-S (serologically negative to IAV-S by IDEXX Influenza A multiscreen ELISA).

On study day 0, piglets were randomized within litter for assignment to 3 treatment groups (generalized block design). Forty-two pigs were administered a single dose of either FluSure XP (n=21) or a placebo control (5% Amphigen diluent, n=21), thus initiating the 4-week 'vaccination phase' of the study (Figure 1). Vaccine treatments were administered as a 2-mL IM injection in the left neck. A second 2-mL IM vaccination was administered 2 weeks later in the right neck (day 14). Three additional non-vaccinated and non-challenged pigs were monitored through day 27 and necropsied for detection of any pre-challenge IAV-S or other endemic disease exposure in the study population (none detected; pigs not included in analyses).

Two weeks after the second vaccination, the 'challenge phase' of the study involved intra-tracheal dosing of each pig on study day 28 with 4 mL of an inoculum containing A/swine/Indiana/A01271853/2012 (H3N2) field isolate acquired from the National Veterinary Services Laboratory (Ames IA). Sequencing and subtyping

Figure 1. Study design and time flow.

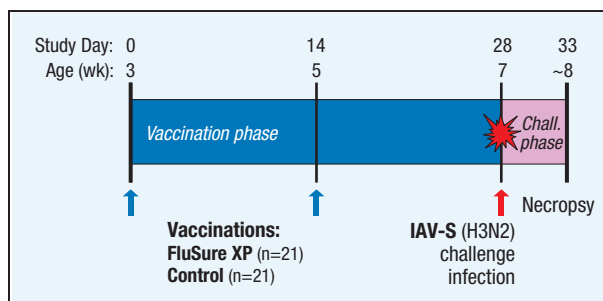
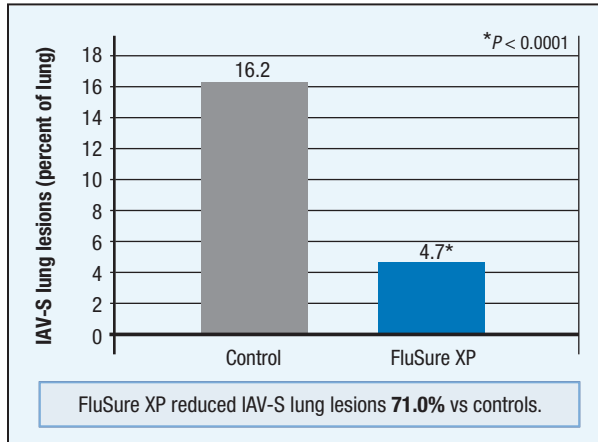


Figure 2. Percent of lungs with IAV-S lesions (LS means).

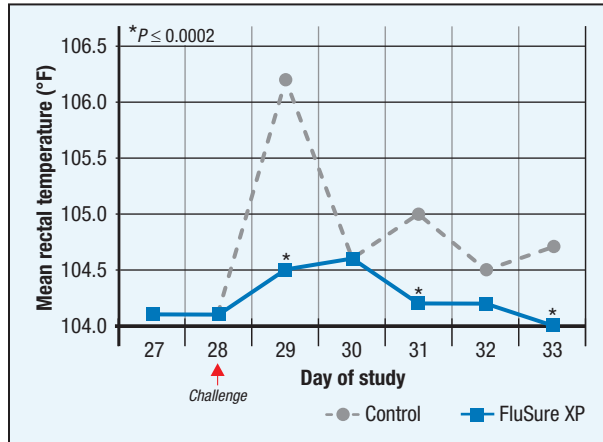


confirmed the heterologous challenge virus was a Cluster IV H3N2, grouping genetically with the Cluster IV-A viruses. The neuraminidase gene was most similar to A/swine/Ontario 55383/04, and the M gene was derived from pH1N1. Based on amino acid similarity, the HA gene of the challenge virus was 97.3% similar to the H3N2 Isolate NC/394 fraction contained in FluSure XP (analysis by UMN VDL).

Challenged pigs were euthanized 5 days post-challenge (day 33) to allow scoring of IAV-S lung lesions, the primary study outcome of interest (percent of consolidation for each lung lobe). Secondary parameters assessed pre-challenge and for 5 days post-challenge included rectal temperatures, virus isolation from nasal swabs, and clinical signs of respiratory disease. In addition, sera were collected before vaccination, before challenge, and at necropsy for measurement of hemagglutination inhibition (HI) antibody titers, and bronchioalveolar lavage (BAL) fluids were collected at necropsy for virus isolation. Clinical assessments and data collections/evaluations were conducted by personnel without knowledge of treatment group assignments.

Data were statistically analyzed by appropriate methods using each pig as an experimental unit. Least squares (LS) means (back-transformed

Figure 3. Mean rectal temperature of pigs before and after IAV-S challenge.



where appropriate) and 95% confidence intervals or ranges were calculated for each treatment, with differences assessed at the 5% level of significance ($P \le 0.05$). The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

Results

Pig vaccinated with FluSure XP and subsequently challenged with the Cluster IV-A H3N2 virus demonstrated the following beneficial outcomes:

■ **Reduced lung lesions:** Figure 2 shows that the mean percentage of lung with lesions at necropsy was significantly ($P \le 0.0001$) reduced in FluSure XP vaccinates (4.7%) compared to the control group (16.2%), representing 71.0% lesion reduction in pigs vaccinated with FluSure XP. Lesions affected <5% of the lung in 45% of FluSure XP vaccinates while 95% of controls had $\geq 5\%$ lung involvement.

■ **Reduced rectal temperature:** Vaccinates had significantly lower mean rectal temperatures than controls on 3 of 5 post-challenge days ($P \le 0.0002$, Figure 3). This treatment effect was apparent even though high baseline temperatures were observed for study pigs (including pre-challenge), likely due to other factors such as excitement/stress during procedures.

Figure 4. Pigs positive for IAV-S in nasal secretions or BAL fluids at any time during study.

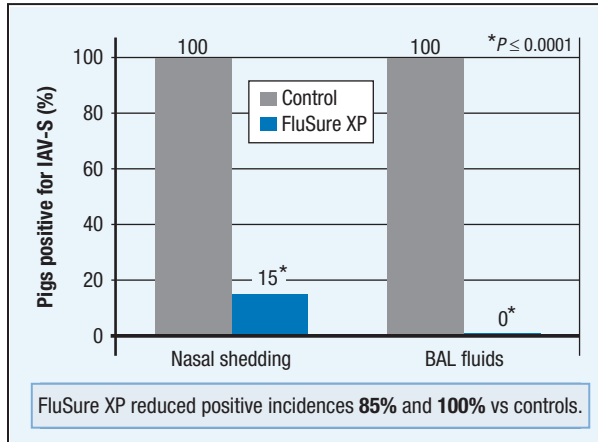
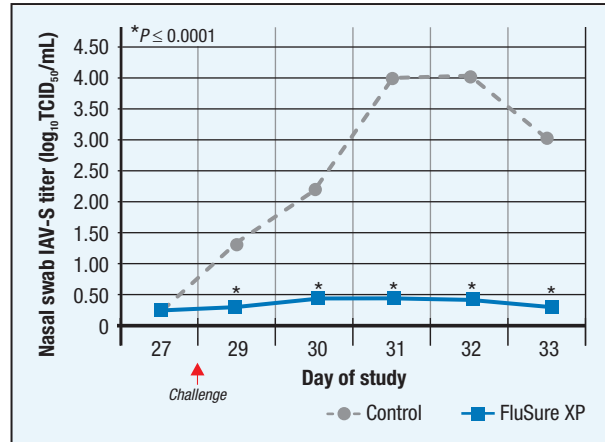


Figure 5. Nasal swab IAV-S titers (LS means; < 0.079 = negative).

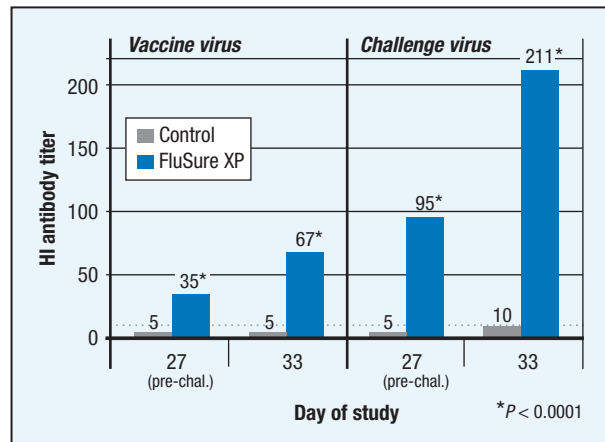


■ **Reduced nasal shedding of IAV-S:** Analysis of nasal swabs collected post-challenge (Figure 4) revealed that the percent of pigs nasal-positive for IAV-S was significantly ($P \leq 0.0001$) reduced in FluSure XP vaccinates (15%) compared to the control group (100%), representing 85% reduction of shedding incidence for pigs vaccinated with FluSure XP. Vaccinated pigs also demonstrated significantly lower mean nasal virus titers on all 5 post-challenge days (29-33) compared to control pigs ($P \leq 0.0001$, Figure 5).

■ **Reduced viral isolation from BAL fluids:** IAV-S titers of BAL fluids were negative for all pigs vaccinated with FluSure XP, but 100% of the control pigs were positive for virus isolation in BAL fluids ($P \leq 0.0001$, Figure 4).

■ **HI titer cross-reactivity:** HI antibody titers were negative to all vaccine viruses and the challenge virus in all study animals on day 0. With the exception of 1 animal, all pigs in the control group maintained negative HI antibody titers to the challenge H3N2 virus throughout the study. In contrast, FluSure XP vaccinates demonstrated positive HI antibody titers ($P < 0.0001$) to vaccine and challenge virus at both day 27 (14 days after the second vaccination but before

Figure 6. HI antibody titers to IAV-S (LS means; titers < 10 = negative).



challenge) and day 33 (5 days post-challenge) (Figure 6). Titers to challenge virus were present in 95% and 100% of vaccinates on days 27 and 33, respectively, compared to 0% and 4.8% of control pigs. These serologic outcomes suggest that FluSure XP vaccination induced active cross-reactive antibody responses to the Cluster IV-A H3N2 challenge strain, indicative of antigenic relatedness between the vaccine and challenge viruses.

Conclusions

Results of this challenge study confirmed that pigs vaccinated with FluSure XP received substantial cross-protection against challenge with a contemporary Cluster IV-A H3N2 virus that contained the pH1N1 M gene (representing the most prevalent H3N2 clade currently circulating in US swine). Compared to controls, FluSure XP vaccinates demonstrated significantly lower percentage of lung with lesions, higher HI antibody

titers to challenge virus, lower percentage of pigs positive for nasal shedding, lower percentage of pigs positive for virus isolation from BAL fluids, and lower mean rectal temperatures.

Veterinarians and producers can confidently use FluSure XP to help achieve comprehensive protection against the most important IAV-S subtypes and clusters circulating in US swine herds.

References

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