Project 4: Efficacy of Selected H1N1 Vaccines in Pigs

Purpose of study:

Following April 2009, when the novel pandemic A/H1N1 virus was detected in the human population of North America, it was not known whether North American pigs had any cross-protective immunity as a result of prior exposure to endemic Swine influenza A viruses (SIV) or SIV vaccines used in the U.S.

Vaccines against 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 addition, in recent years, use of autogenous vaccines 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. In the U.S., autogenous 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 can only be used in the herd of origin. Most autogenous vaccines are also multivalent with various combinations of H1 and H3 subtypes.

Here we report the efficacy of vaccinating weaned pigs with one of 3 different inactivated vaccines produced from endemic North American SIV and compared their immune reactions to that achieved with a homologous inactivated monovalent vaccine produced from a strain of the 2009 A/H1N1 virus.

Experiment:

- Six groups of 10 pigs, free of SIV and porcine reproductive and respiratory syndrome virus (PRRSV) were housed in biosafety level 2 (BSL2) containment until immunity developed after vaccination. Immediately prior to being challenged with A/California/04/2009 H1N1 (CA/09), pigs were transferred to ABSL3 containment. Pigs were vaccinated intramuscularly at approximately 4 weeks of age, boosted at 7 weeks of age, and challenged at 10 weeks of age. Pigs were cared for in compliance with the Institutional Animal Care and Use Committee of the NADC-USDA-ARS.

- Three vaccines from commercial manufacturers were selected based on serologic evidence of cross-reactivity in a previous study and administered as per label or manufacturer recommendation.

    - Vaccine A (FluSure® XP, Pfizer Animal Health, New York, NY) - a fully licensed trivalent commercial product containing cluster IV H3N2, γ-cluster H1N1, and δ-cluster H1N1 SIV.

    - Vaccine B (MaxiVac Excell® 5.0, Intervet/Schering-Plough, Boxmeer, The Netherlands) - a pentavalent product under review for full licensure containing clusters I and IV H3N2 and β-, γ-, and δ-cluster H1 SIV.

    - Vaccine D (Newport Labs, Worthington, MN) - a bivalent autogenous vaccine containing β- and γ-cluster H1 SIV.

- In addition, a monovalent experimental vaccine (Vaccine E) was prepared from CA/09 at 8 HA units per 50 µL and 1 X $10^{6.5}$ 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 were challenged intratracheally with 2 mL of 1 x 10^5 $TCID_{50}$ of CA/09 and observed daily for signs of clinical disease and fever. Nasal swabs were taken on 0, 3, and 5 days post infection (dpi) to evaluate nasal virus shedding. Pigs were humanely euthanized at 5 dpi to evaluate lung lesions and viral load in the lungs.

- Hemagglutination inhibition (HI) titer assays were 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.

Results:

- Two doses of the homologous CA/09 vaccine provided optimal immunity against 2009 A/H1N1 challenge in all parameters evaluated:
    - Prevented nasal shedding in all pigs challenged with CA/09 A/H1N1 at both time points after challenge.
    - No virus detected from the lungs of any pigs vaccinated with the homologous vaccine.
    - Significantly reduced rectal temperatures versus non-vaccinated challenged controls during the acute 5-day post-infection period, including protection from the peak febrile response at 24 hours post infection.
    - Complete protection from macroscopic and microscopic pneumonia.
    - Protection was associated with robust HI antibody titers specific to 2009 A/H1N1.
    - Induction of cross-reacting HI antibodies to the γ-cluster SIV OH/07 and significant titers of cross-reacting antibodies to the α-cluster MN/99 and β-cluster NE/08.

- There were statistically significant reductions in average 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, in both groups, virus was detected in the nose or lung.

- Vaccines A, B and D demonstrated statistically significant reduction in lung lesion scores versus non-vaccinated challenge controls.

- The three commercial vaccines failed to prevent elevated rectal temperatures compared to non-challenged control pigs 24 hrs following challenge. Although there was no

significant difference in protection against the febrile response between any of the three commercial vaccines tested, the autogenous vaccine had a significantly reduced febrile response compared with the non-vaccinated challenge controls.

- Average CA09 HI antibody titers of pigs vaccinated with any of the commercial vaccines were below the detectable threshold or positive cut-off.

- By ELISA assay, only vaccines D and E induced levels of IgG significantly higher than negative controls against the 2009 A/H1N1 virus in the serum or BALF.

Conclusions:

- The experimental infections in nonvaccinated challenged control pigs were characteristic of acute influenza illness in swine. Clinical signs, pathologic changes and virus replication were restricted to the respiratory tract.

- Significant protection from virus replication and shedding was demonstrated with all three of the vaccines. In particular, the homologous monovalent vaccine induced optimal protection by complete prevention of viral shedding from 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 the 2009 A/H1N1 vaccine anti-sera, suggesting robust titers against γ-cluster H1 SIV in pigs may be correlated with partial cross-protection against pandemic A/H1N1.

- Based on cross-protection (against lung lesions) demonstrated with the vaccines evaluated in this study, the U.S. swine herd likely has some degree of immunity to the 2009 A/H1N1 from prior vaccination or natural exposure to viruses inducing cross-reacting antibodies. 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. Based on results demonstrated here, consideration should be given for development of monovalent homologous vaccines to be used in all age groups of naïve pigs to best protect the swine population and to limit the potential transmission of 2009 A/H1N1 among pigs or from pigs to people.

Note: Autogenous vaccines have the advantage that they can be produced relatively quickly with contemporary circulating viruses 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 scientific peer-reviewed information is publically 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. 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.