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SWINE INFLUENZA CASE STUDY: H3N2 STRAIN DIFFERENCES AS DETERMINED BY HA SEQUENCING, HI SEROLOGY, AND VACCINATION CHALLENGE

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Introduction and Objectives

Swine influenza virus (SIV) continues to be a significant viral respiratory pathogen affecting swine herds and two subtypes, H1N1 and H3N2 are most frequently isolated from swine during outbreaks. It is well accepted that genetic shift, resulting in a new subtype, occurs, as does genetic drift, a more subtle change in the viral genome. In comparison to human populations, where influenza vaccines are modified annually to remain effective, these changes in swine populations have been thought to be less frequent or significant. Increasing evidence suggests that genetic drift may be increasing in swine, requiring new vaccination and control strategies. In this case study, two H3N2 SIV strains isolated from a large production system were evaluated by genetic sequencing of the hemagglutin (HA) portion of the genome and then compared clinically in two vaccine studies using monovalent vaccines prepared from the two strains. One strain (0108) had been used in an autogenous vaccine, while the other (1776) was a more recent strain from an outbreak. Consideration was given to the advisability of including the recent strain in the vaccination program and these experiments were conducted to attempt to answer that question.

Material and Methods

Both H3N2 strains were submitted to a molecular biology laboratory for HA sequencing. Amino acid sequences were deduced from the nucleotide sequences and the charge and stereochemistry properties of the respective amino acids compared. In addition, and most importantly, amino acid differences at recognized antigenic sites or epitopes were compared. To determine the clinical significance of these differences, two monovalent vaccines were made and HI serology was done post-vaccination using homologous and heterologous antigens.

In a second experiment, three groups of pigs were vaccinated with monovalent vaccines prepared from the 0108, 1776, and another H3N2 strain identified as 7940. Challenge (1776 strain) of vaccinated pigs and a non-vaccinated control group was done and virus shedding determined by Directigen™ testing and virus isolation (VI).

Results

A total of 566 amino acid residues representing the HA of the two H3N2 strains were compared. There were 36 differences or 6.3% of the total. Of the 36 differences, 21 involve differences in character, e.g., polar vs. nonpolar, hydrophilic vs. hydrophobic. Most significant were differences in amino acid residues at antigenic sites. These sites have been designated A, B, C, D for the H3 HA molecule. Amino acid differences between the two viruses' HA's were noted at all four sites. Each site had one amino acid difference, except for "A" which had two. In the first experiment, when vaccinated pigs were tested for HI antibody using homologous SIV antigen, titers of $\geq$ 640 were seen for both 0108 and 1776 vaccinated pigs. HI antibody titers using heterologous antigen resulted in titers of 20-40 (0108 vaccine) and 80-320 (1776 vaccine). In the vaccination-challenge study, nasal swabs were collected from all pigs on days 3, 5, and 7 post-challenge. On day 3, only the 0108 vaccinated group was positive by Directigen™ testing. On day 5 all groups, except the 1776 vaccinated group were positive. On day 7, all groups were negative. Virus isolation correlated with the Directigen™ test results.

Discussion and Conclusion

In this case study, the two H3N2 strains were shown to have a significant number of differences in the deduced amino acids of the HA and amino acid differences at the key antigenic sites of the HA. In addition, the two vaccine experiments demonstrated a difference in immunity against heterologous challenge as determined by HI serology and viral shedding.