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Molecular Characterization of the Hemagglutinin and Neuraminidase Genes from a H3N2 Swine Influenza Virus Isolate Recovered from the United States

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Introduction

The nucleotide sequences of the genes encoding the hemagglutinin (HA) and neuraminidase (NA) proteins were determined for a swine influenza virus (SIV) field strain recently isolated from a herd experiencing respiratory disease in the central United States. Classical hemagglutination-inhibition and neuraminidase-inhibition assays at NVSL confirmed the isolate was H3N2. The molecular characterization of this isolate will be presented and the significance of the results discussed. Additional H3N2 SIV field isolates from Illinois and North Carolina are currently being examined.

Materials and Methods

Virus. The SIV isolate A/swine/Iowa/99 (H3N2) used in this study was isolated from the nasal swab of a piglet from a swine herd in Iowa. The A/Port Chalmers/1/73 (H3N2) virus strain was used as a control. All isolates were propagated in 9-12 day old embryonated eggs or MDCK cells.

Cloning of the H3 and N2 genes. Viral RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Reverse transcription was performed using random primers (Clontech, Palo Alto, CA). PCR primers were designed to amplify the H3 and N2 genes based upon available published DNA sequence.

Nucleotide sequencing. DNA sequencing reactions were performed with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AMPLITAQ™ DNA polymerase (Perkin-Elmer) according to manufacturer's instructions. The sequences obtained were assembled and compared using DNASTar software. GenBank sequence databases were used for homology searches.

Results and Conclusions

Comparison of the H3 genes of A/Port Chalmers/1/73 and A/swine/Iowa/99 revealed a nucleotide identity of 91% and an amino acid similarity of 89%. Searches of the GenBank sequence databases identified the human influenza strain A/Vienna/47/96 as having the highest amino acid similarity (96%) with H3 of A/swine/Iowa/99.

The N2 genes of A/Port Chalmers/1/73 and A/swine/Iowa/99 displayed nucleotide and amino acid identities of 91% and 90%, respectively. The deduced amino acid sequence of N2 of A/swine/Iowa/99 showed the highest degree of similarity (98%) with A/Shiga/25/97, a recent human influenza strain.

Phylogenic analysis, performed using the amino acid sequences of H3 and N2 from various influenza isolates, showed that A/swine/Iowa/99 more closely resembles current human influenza strains than earlier swine H3N2 viruses and human viruses from the mid 1970s.