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GENETIC AND SEROLOGICAL CHARACTERIZATION OF H1N1 SWINE INFLUENZA VIRUSES

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Introduction

Until 1998 when H3N2 swine influenza (SI) viruses first emerged, the predominant SI viruses circulating within the United States were classical swine H1N1. Recently, antigenically and genetically distinct H1N1 and H1N2 viruses have been identified, a result of both antigenic drift and genetic reassortment (2,4). This study examined the genetic and serologic diversity of recent H1N1 isolates compared to classical H1N1 (cH1N1).

Materials and Methods

SI Virus Isolates: Two H1N1 viruses, designated IAa and IAb, isolated from the same farm in Iowa in 1999, were provided by K-J Yoon, IVDL, Ames, IA. Five H1N1 isolates, designated NCa through NCe were obtained from four different nursery and finishing farms in North Carolina in 2000. NCc was identified as a reassortant H1N1 (rH1N1) virus by Dr. R. Webby, St. Jude Children's Research Hospital, Memphis, TN.

Hemagglutination Inhibition (HI) Testing: Antisera were prepared by vaccinating SI-negative swine with inactivated, adjuvanted monovalent vaccines prepared from isolates NCc, NCd, and NCe. Sera were tested for HI antibodies to inactivated antigen preparations using the kaolin procedure. Positive controls included H1N1 and H3N2 vaccine sera and reference cH1N1 serum from the NVSL, Ames, IA. Sera were also tested using the HerdCheck® H1N1 SI Virus Antibody Test Kit (IDEXX Laboratories, Inc., Westbrook, ME)

Cloning and Sequencing of the HA genes. Extraction of viral RNA, reverse transcription, and PCR were performed as described previously (1). Sequencing was performed by the Microchemical Core Facility, San Diego State University. HA sequences were compared to those for the H1N1 vaccine strain and classical A/Swine/WI/94/U5316, obtained from GenBank.

Results and Discussion

Genetic Homology: IAa and IAb, isolated from the same herd, were genetically distinct (Table 2). IAa was most similar to cH1N1 viruses while IAb was genetically distinct from both cH1N1 and the 2000 NC viruses. NCd was most similar to rH1N1 NCc, indicating that NCd is likely also a rH1N1.

Table 2: Percent Identity of the H1N1 HA gene

Isolate	WI94	IAa	IAb	NCa	NCc	NCd
Vacc	98.3	97.2	94.3	94.3	94.9	93.8
WI94		96.6	94.9	93.8	93.8	92.6
IAa			93.2	93.8	92.6	91.5
IAb				91.5	89.6	88.6
NCa					90.9	90.3
NCc						98.9

Serological Cross-reactions: Sera from pigs generally developed the highest HI titers to homologous antigen; however, there was cross-reactivity for all H1N1 antigens tested (Table 3). The NVSL sera recognized antigen from all viruses with high titers. IDEXX antibodies did not distinguish among the H1N1 isolates as all pigs developed positive titers (data not shown).

Table 3: HI serological cross-reactions of H1N1 isolates

	Test Antigen				
Sera	NCc	NCd	NCe	Vacc	H3N2
NCc	80	160	160	40	neg
NCd	320	≥ 640	≥ 640	320	neg
NCe	20	40	320	40	neg
Vacc	10	20	40	160	neg
NVSL	160	320	≥640	≥640	neg
H3N2	neg	neg	neg	neg	80

Shaded cells are homologous titers.

Summary. In spite of considerable genetic diversity among recent field isolates, HI cross-reactions were apparent. Since adjuvant and antigenic dose are factors in the ability of vaccines to protect against heterologous H1N1 virus isolates (3) controlled challenge studies are required to determine if a vaccine cross-protects against these viruses. The observed genetic diversity does indicate a need to evaluate candidate viruses for inclusion in vaccines that would offer the broadest spectrum of protection against the H1N1 viruses presently co-circulating within swine herds in the United States.

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