RECENT OBSERVATIONS OF SWINE INFLUENZA DISEASE AND PROPHYLAXSIS IN US SWINE HERDS

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Introduction . Influenza in swine is caused by a segmented, negative sense RNA virus of the family *Orthomyxoviridae*[1]. World wide there are 14 different HA protein types and 9 different NA proteins, all are found in avian species, but not all are capable of infecting mammals[2]. Classically US swine have been infected by the H1N1 subtype, but recently (1998) the introduction of the H3N2 subtype and the reassortment virus H1N2 (1999) has complicated vaccination and management strategies.

Influenza viruses persist in a population by the well-documented processes of antigenic drift and antigenic shift. Antigenic shift occurs when an animal undergoes dual infection by more than one subtype and the virus reassorts gene segments and develops a new combination of HA and NA envelope proteins. We have recently identified reassortment H1N2 viruses.[3,4] Antigenic drift occurs by random point mutations and single amino acid substitutions in the HA and NA envelope proteins. Antigenic drift has been observed in human populations at a rate of 1% per year,[1] or about 5 to 6 nucleotides per year[5]. Traditionally, antigenic drift of H1N1 swine viruses has thought to occur at a much slower rate because of a large population of naive animals and the lack of vaccination immune pressure.[6]
Recently a report by Thacker [7] and numerous reports from diagnostic laboratories have shown an inconsistent, mixed or poor seroconversion to H1N1 SIV by animals vaccinated with autogenous swine influenza vaccines. Also recent conversations with diagnosticians have indicated clinical and histological evidence of SIV infection but failure of seroconversion to SIV in convalescent sera. We sought to explain these observations by comparing the virus neutralization immune responses (HI) of pigs following vaccination with a bivalent H1N1/ H3N2 SIV autogenous vaccine which incorporated recent isolates. The porcine sera were evaluated by HI responses to classic H1N1(1973), recent H1N1(2000), and recent H1N2 (2001) SIV isolates. We also evaluated porcine sera using a commercial H1N1 ELISA test kit. The viruses were further investigated by sequencing portions of their viral HA proteins.

Materials and Methods. Experiment 1. Forty-five clinically normal, mixed gender pigs approximately 6 weeks of age and considered to be seronegative to SIV subtypes H1N1 and H3N2 were used in this study. Pigs were ear-tagged, commingled, housed in a controlled environment, and fed standard rations throughout the trial. Pigs were acclimated for 7 days prior to initiation of the trial. Animals were randomly divided into two groups: 30 vaccinates and 15 nonvaccinated sentinel controls. Vaccinated animals received a bivalent SIV vaccine containing SuprImmâ oil adjuvant. Vaccine virus was propagated in mammalian cells. Pigs were vaccinated on days 0 and 14 of the study and bled on days 14 and 35. HI assays were performed on all sera using an Iowa State University standardized protocol. HI assays were performed using 2 different H1N1 isolates, an isolate homologous to the vaccine virus and a heterologous NVSL A/SW/IA/73 isolate, and a recent 2001 H1N2 isolate A/SW/IA/01. Experiment 2. In a separate experiment we measured the serologic responses of 30 field vaccinates to homologous H1N1 and H3N2 viruses and heterologous H1N1 virus using the IDEXX ELISA test kit. Influenza virus sequencing was blinded and performed by Dr. Richard Webby, Dept. of Virology and Molecular Biology, St. Judes Children's Hospital. Serological data were compared using a one-way analysis of variance. If significant, pairwise comparisons of the treatments were performed by least significant differences. Statistical significance for all tests was set at $p<0.05$.

Results and Discussion. Geometric mean HI titers and statistical comparisons are shown in Table 1. Lack of seroconversion to any of the tested SIV subtypes by sentinels confirms the lack of field virus exposure during the study period. Vaccinates showed a significant (4-fold or greater) seroconversion to homologous H1N1 SIV virus after 2 vaccinations. However, these same sera showed significantly lower (4-fold or greater) viral neutralizing HI responses to the heterologous diagnostic laboratory H1N1 isolate. The virus neutralizing immune response to the heterologous

H1N2 virus as measured by HI titer was variable and pig dependent, with titers ranging from 2-fold to 32-fold lower than the response to the homologous vaccine H1N1 virus isolate. The geometric mean titer of the sera was significantly lower to the H1N2 isolate (170.9 vs 522.7) as compared to the homologous H1N1 vaccine isolate. Experiment 2. Sera of 30 autogenous field vaccinates had 2/30 positive by ELISA and 1/30 with HI titer of 40 or above using heterologous H1N1 analysis, but 27/30 with HI titer of 40 or above and a geometric mean titer of 95.6 using homologous H1N1 analysis (Table 3). Genetic sequencing of the three SIV subtypes supports the differences seen in the serological responses of these animals (Table 2). The 2 recent H1N1 isolates (A/SW/NC/1C806/01and A/SW/NC/0D375/00) and the H1N2 virus (A/SW/IA/1D930/01) have only 80% amino acid sequence homology to the HA protein of the 1973 H1N1 isolate (A/SW/1H186/73) used in many diagnostic laboratories, whereas the two H1N1 isolates from years 2000 and 2001 are 97% homologous. Likewise, the H1N2 isolate was only 82% homologous to the 1973 H1N1 isolate and was greater than 5% different from the 2 recent H1N1 isolates.

The serologic HI titers and the genetic sequence analysis of the HA proteins of the 3 different H1N1 SIV isolates and the H1N2 SIV isolate support the conclusion that significant antigenic drift has occurred in the HA envelope protein antigen of US H1 SIV subtypes over the time period from 1973 to 2001. Results also indicate that serologic analysis of H1 subtypes using the 1973 H1N1 isolate does not accurately represent responses to vaccination with current autogenous vaccines. It may also indicate that serological results from diagnostic laboratories may not accurately reflect the status of swine infected by recent H1N1or H1N2 SIV isolates. Practitioners using autogenous vaccines need to be aware that there is often an apparent lack of detectable immune responses by current diagnostic HI tests. This may falsely lead to the vaccination of piglets carrying significant interfering maternal antibodies and cause subsequent vaccine failures. It appears from the genetic sequencing of the HA envelope protein of the H1N2 isolate compared to the H1N1 isolates that its HA protein is different from the recent H1N1 isolates and may represent a stable lineage of virus capable of cocirculation with current H1N1 and H3N2 isolates. Such a conclusion is consistant with recent data[9]. It is not known whether the current commercial ELISA test would allow a more accurate diagnosis of immune status; however, preliminary field reports from herds using autogenous vaccines indicate that vaccination titers may not be accurately reflected using the ELISA test. It is also not known if clinical infections caused by recent H1N1and H1N2 SIV isolates are detected by this ELISA. It has yet to be determined whether the current commercial vaccines adequately protect animals from infection by the recent H1N2 isolates; however, a European prototype vaccine containing H1N1 did not protect against a heterologous H1N2 challenge.[8]

Current Prophylaxis and Diagnosis. Numerous and everchanging methods have been employed to try to keep pace with the rapidly evolving swine influenza virus in US swine populations. A H1N1 isolate (A/SW/IA/73) is currently used by NVSL and numerous US diagnostic laboratories for evaluation of seroconversion to SIV via a hemagglutination inhibition (HI) assay. The HI assay has been considered the "Gold Standard" for determining strain-specific serological responses of swine to Swine Influenza. An antigen capture ELISA, Directogen Ô by Becton Dickenson, is also available commercially in the US. This assay is specific for all type A influenza viruses of human or animal origin and is based on the matrix protein of the virus. Currently an indirect ELISA assay is being marketed by IDEXX to evaluate seroconversion to H1N1 SIV, and an indirect H3N2 assay developed by the same company is being evaluated by diagnostic laboratories. Several diagnostic laboratiories are offering PCR based typing of H1N1, H3N2, and H1N2 viruses. In a recently published study[9], multiplex PCR data has demonstrated that the H1N2 isolate has been identified in approximately 4% of Midwest diagnostic SIV isolates and has been found in 9 different states. These data indicate that the virus has been present since 1999 and is spreading rapidly in US herds.

In 1994, Syntrovet introduced a H1N1 swine influenza virus (SIV) vaccine (MaxiVac-Fluâ now marketed by Schering Plough) using a H1N1 isolate. This vaccine was the first federally approved SIV vaccine for commercial use. Bayer introduced a second commercially approved H1N1 SIV vaccine in 1997. In 1998 the H3N2 virus was introduced into the US swine population. Autogenous vaccine manufactures quickly began producing herd specific vaccines containing H3N2 isolates in response to this disease introduction. In 1999 Schering Plough introduced the first conditionally approved H3N2 SIV vaccine. This vaccine contained a H3N2 virus strain obtained

from Europe (Port Chalmers). Although the virus was found to cross-react with US H3N2 strains, it did not induce significant neutralizing HI antibodies to US isolates and a second H3N2 SIV isolate (Midwestern-US) was added to this conditionally approved vaccine. InterVet licensed the first commercially approved bivalent (H1N1, H3N2) SIV vaccine in 2001 and Schering recently licensed a bivalent SIV vaccine in 2002. Autogenous vaccine manufacturers continue to produce monovalent, bivalent, and trivalent SIV vaccines.

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Table 1. Geometric Mean HI Titer

	IT H1N1 (DO)	IT H1N1 (D35)	NVSL H1N1 (DO)	NVSL H1N1 (D35)	IT H1N2 (DO)	IT H1N2 (D35)
C	31.3 (a)	32.0 (a)	22.0 ©	23.3 ©	8.46 (e)	12.31 ©
V	40.7 (a)	522.7 (b)	20.3 ©	62.7 (d)	9.05 (e)	170.95 (f)

Table 2. Genetic Sequencing of SIV Isolates

Identity (%) of first 480 bp of HA open reading frame

	Sw/IA/1H186/73	Sw/NC/1C806/01	Sw/NC/OD375/00	Sw/IA/1D930/01
Sw/IA/1H1H186/73	100			
Sw/NC/1C806/01	81.5	100		
Sw/NC/OD375/00	82.7	97.9	100	
Sw/IA/1D930/01	82.5	92.1	93.3	100

Identity (%) of first 160 amino acids HA open reading frame

	Sw/IA/1H186/73	Sw/NC/1C806/01	Sw/NC/OD375/00	Sw/IA/1D930/01
iSw/IA/1H1H186/73	100			
Sw/NC/1C806/01	80.5	100		
Sw/NC/OD375/00	81.8	96.9	100	
Sw/IA/1D930/01	81.1	92.5	94.3	100

Identify (%) of last 770 bp of HA open reading frame

	Sw/IA/1H186/73	Sw/NC/1C806/01	Sw/IA/D930/01
Sw/IA/1H1H186/73	100		
Sw/NC/1CH806/01	81.0	100	
Sw/IA/1D930/01	83.0	91.6	100

Identify (%) of last 256 amino acids of HA open frame reading

	Sw/IA/1H186/73	Sw/NC/1C806/01	Sw/IA/D930/01
Sw/IA/1H1186/73	100		
Sw/NC/1C806/01	79.3	100	
Sw/IA/1D930/01	82.0	89.0	100

Table 3. Comparison of ELISA and HI Titers

						Diagnostic laboratory		ImmTech laboratory	
		IDEX ELISA Titer (H1N1)				Heterologous HI Titers		Homologous HI Titers	
Tube #	ID #	S/P Ratio	Titer	Titer Group	Result	H1N1 Titer	H3N2 Titer	H1N1 Titer	H3N2 Titer
1	Y11	0.095	176	0	Neg	Neg	40	ND	ND
2	Y25	0.006	9	0	Neg	Neg	Neg	40	20
3	B2135	0.128	244	0	Neg	20	40	320	640
4	B1882	0.064	114	0	Neg	Neg	10	40	20
5	B2129	0.239	481	0	Neg	10	160	160	1280
6	B2167	0.425	901	1	Pos!	10	160	80	1280
7	B1730	0.193	381	0	Neg	10	40	160	320
8	B1334	0.046	80	0	Neg	Neg	20	20	10
9	B1526	0.141	271	0	Neg	Neg	160	80	640
10	G457	0.107	200	0	Neg	Neg	10	40	40
11	B1589	0.266	541	0	Neg	10	40	80	320
12	B1492	0.349	727	0	Neg	20	160	640	1280
13	B1109	0.012	18	0	Neg	Neg	20	40	40
14	B6	0.315	650	0	Neg	20	80	160	320
15	B2173	0.398	839	0	Neg	20	40	640	640
16	B2124	0.128	244	0	Neg	10	40	160	640
17	B1784	0.352	734	0	Neg	Neg	40	80	160
18	G494	0.076	138	0	Neg	Neg	20	80	160
19	B1586	0.22	440	0	Neg	20	160	320	640
20	B1731	0.22	440	0	Neg	10	80	160	640
21	B1652	0.382	803	0	Neg	20	40	80	320
22	B1825	0.168	328	0	Neg	Neg	40	80	160
23	B1814	0.08	146	0	Neg	10	10	20	20
24	B1835	0.086	158	0	Neg	Neg	10	40	40
25	G265	0.636	1399	1	Pos!	40	20	160	320
26	B1566	0.107	200	0	Neg	10	20	80	160
27	B1726	0.291	597	0	Neg	10	20	320	640
28	B1874	0.061	109	0	Neg	10	10	40	80