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Characterization of H3N2 Swine Influenza Viruses in Iowa Swine

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Abstract
Since December 1998 when swine influenza virus (SIV) with H3N2 was first identified in Iowa swine, prospective and retrospective studies were conducted to monitor and evaluate H3N2 SIV infections in swine population in Iowa until February 2000. A serological survey revealed that H3 SIV had been widely spread in Iowa swine within first 6 months after initial identification and that both H1 and H3 SIV coexisted. Many herds tested had the evidence that animals were exposed to both subtypes of SIV. In some cases, both subtypes were isolated from the same animal. All circumstantial evidences strongly suggested the emergence of new subtype due to reassortment of H1N1 and H3N2 strains; however, no evidence of new reassortant was yet found in Iowa during this study period. A one-way hemagglutination inhibition (HI) assay on banked field isolates of H3N2 SIV demonstrated that the majority of field isolates were antigenically conserve. These observations suggest that diagnostic assays using an initial Midwest H3N2 SIV isolate should be reliable for diagnosing infection of H3N2 SIV. This observation also suggests that a vaccine using an initial Midwest H3N2 SIV may provide reliable cross protection against a variety of H3N2 strains. However, a few isolates were found resistant to HI activity conferred by antiserum raised against a H3N2 Midwest isolate, warranting a continuous surveillance for antigenic drift among isolates.

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Characterization of H3N2 Swine Influenza Viruses in Iowa Swine

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Summary and Implications
Since December 1998 when swine influenza virus (SIV) with H3N2 was first identified in Iowa swine, prospective and retrospective studies were conducted to monitor and evaluate H3N2 SIV infections in swine population in Iowa until February 2000. A serological survey revealed that H3 SIV had been widely spread in Iowa swine within first 6 months after initial identification and that both H1 and H3 SIV coexisted. Many herds tested had the evidence that animals were exposed to both subtypes of SIV. In some cases, both subtypes were isolated from the same animal. All circumstantial evidences strongly suggested the emergence of new subtype due to reassortment of H1N1 and H3N2 strains; however, no evidence of new reassortant was yet found in Iowa during this study period. A one-way hemagglutination inhibition (HI) assay on banked field isolates of H3N2 SIV demonstrated that the majority of field isolates were antigenically conserve. These observations suggest that diagnostic assays using an initial Midwest H3N2 SIV isolate should be reliable for diagnosing infection of H3N2 SIV. This observation also suggests that a vaccine using an initial Midwest H3N2 SIV may provide reliable cross protection against a variety of H3N2 strains. However, a few isolates were found resistant to HI activity conferred by antisera raised against a H3N2 Midwest isolate, warranting a continuous surveillance for antigenic drift among isolates.

Introduction
Influenza is a common respiratory disease of swine in most of the swine-producing regions throughout the world. Since 1918, when the first cases of swine influenza were discovered in the United States (3), the predominant subtype that has affected pigs in the United States has been the classic H1N1 (2). This strain is similar genetically and antigenically to the type A virus implicated in “Spanish Flu” (4). However, since December 1998 and January 1999, a H3N2 subtype of swine influenza virus (SIV) was isolated from clinically affected swine in Iowa (IA), Minnesota, North Carolina (NC), and Texas (TX). A genetic analysis demonstrated that Midwest isolates (IA, MN, TX) and NC isolate were distinct (5). Herein, prospective and retrospective observational studies were conducted on Iowa swine to 1) assess the prevalence of H3N2 SIV infections; 2) determine/monitor the emergence of NC genotype and new reassortant; and 3) characterize the antigenic stability of H3N2 SIV isolates.

Materials and Methods
For surveillance, all SIV isolates obtained since December 1998 were continuously subtyped by a PCR-based differential test developed in Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) and/or an immunological assay at the National Veterinary Services Laboratories. In June 1999, 6 months after initial detection of H3N2 in Iowa, a total of 1,604 sera was collected from 6-month-old finishers in 129 swine operations throughout 29 counties in Iowa and tested by hemagglutination inhibition (HI) test for antibodies to both H1N1 (A/Sw/IA/30) and H3N2 (A/Sw/IA/41305/98, A/Sw/NC/35922/98) viruses.

Once a bank of H3N2 SIV isolates (N=150) was collected at ISU-VDL, the potential for antigenic drift in HA protein among isolates was assessed by one-way HI test using two polyclonal antisera each of which was raised against one of initial H3N2 Midwest strains (A/Sw/TX/4199-2/98) and NC strain (A/Sw/NC/35922/98), respectively. Isolates represented cases submitted to ISU-VDL between January 1999 through February 2000.

Results and Discussion
Influenza outbreaks by H3N2 SIV were initially identified in swine operations at northwestern part of Iowa and quickly spread to herds in other parts of the state. In June, the serological survey on finishing pigs revealed that 64% of pigs and 92% of herds were serologically positive for the Midwest H3N2 virus, indicating that H3N2 SIV was already widespread throughout the Iowa swine population. About 30% of swine tested had antibodies for both H1 and H3 SIV, showing that both strains were circulating in swine herds at the same time. It suggested the risk for the emergence of new subtype(s) due to reassortment between H1N1 and H3N2. However, neither new reassortant nor NC genotype was identified during this study. Antigenically, hemagglutinin (HA) of the majority (96.7%) of H3N2 SIV isolates tested appeared to be stable; however, a few isolates (N=4) were significantly resistant to HI activity by both TX and NC antisera.

Collectively, these results demonstrate that H3N2 strains of SIV are of significance to the veterinary profession. Our observations also indicate that both H1 and H3 subtypes will co-exist as significant pathogen. High seroprevalence of H3N2 in Iowa justifies the need for an effective vaccine to control swine influenza, as was the case for H1N1 SIV. The relatively good antigenic stability of HA protein among field isolates suggests that vaccines and diagnostic tests using an initial Midwest H3N2 SIV isolate should provide reliable cross-protection against H3N2 strains and be reliable for diagnosing the respiratory disease by H3N2 SIV, respectively.

Nevertheless, a continuous surveillance for the emergence of new subtype(s) should remain effective. Identification of a few HI resistant isolates warrants further investigation on the antigenic drift among isolates as it was the case in Europe (1).
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