Evaluation of transmission of swine influenza type A subtype H1N2 virus in seropositive pigs

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Objective—To examine clinical signs, virus infection and shedding, and transmission of swine influenza virus (SIV) subtype H1N2 among seropositive pigs.

Animals—Eighteen 3-week-old pigs with maternal antibodies against SIV subtypes H1N1, H3N2, and H1N2.

Procedure—Ten pigs (principal) were inoculated intranasally with subtype H1N2 and 2 groups of contact pigs (n = 4) each were mixed with principal pigs on day 7 (group 1) or 28 (group 2). Two principal pigs each were necropsied on days 4, 14, 21, 28, and 42 days after inoculation. Four pigs in each contact group were necropsied 35 and 14 days after contact. Virus excretion was evaluated after inoculation or contact. Lung lesions and the presence of SIV in various tissues were examined.

Results—Mild coughing and increased rectal temperature were observed in principal pigs but not in contact pigs. Nasal virus shedding was detected in all principal pigs from day 2 for 3 to 5 days, in group 1 pigs from day 2 for 4 to 9 days after contact, and in group 2 pigs from day 4 for 2 to 6 days after contact. Trachea, lung, and lymph node specimens from infected pigs contained virus. Antibody titers against all 3 subtypes in all pigs gradually decreased.

Conclusions and Clinical Relevance—Protection from viral infection and shedding was not observed in pigs with maternal antibodies, but clinical disease did not develop. Vaccination programs and good management practices should be considered for control of SIV subtype H1N2 infection on swine farms. (Am J Vet Res 2004;65:303–306).

Swine influenza virus (SIV) is one of the most common respiratory tract pathogens in pigs. Clinical signs include high fever, lethargy, nasal discharge, coughing, dyspnea, and weight loss. Although SIV alone may not be clinically important, the virus commonly interacts with other bacteria and viruses, causing major economic losses. Worse respiratory tract disease has occurred since the emergence of new SIV subtypes H3N2 and H1N2 in the US swine population. Swine influenza virus is also a zoonotic disease, and transmission of the virus from pigs to humans has been well documented.1-5

Various subtypes of type A influenza virus have been reported from humans and other animals, and their characterization is based on differences in the hemagglutinin (HA) and neuraminidase genes.6,7 Presently, 3 subtypes (H1N1, H1N2, and H3N2) are commonly known in the pig population throughout the world. In the United States, classical H1N1 subtype was exclusively prevalent until 1998.1,8 However, H3N2 viruses with genes derived from human, swine, and avian viruses have become evident since 1999. Shortly after the appearance of H3N2 virus, H1N2 viruses that resulted from reassortment between the triple reassortant H3N2 and classical H1N1 viruses have also been isolated from pigs in at least 9 different states; > 10% of the SIV field isolates tested in 2001 belonged to H1N2 subtype.1

Movement of pigs from infected to susceptible herds is believed to be one of the main routes of SIV transmission between farms. Within a farm with infected pigs, the primary route of transmission is through pig-to-pig contact via the nasopharyngeal route because virus from infected pigs is excreted through nasal secretions and disseminated by aerosols. Different stress factors including poor ventilation and temperature fluctuation can enhance the spread of influenza viruses. Clinical outbreaks of the disease can occur throughout the year but are usually more common during the colder months.1,9 Once a herd becomes infected, the virus is likely maintained within the farm because of the continuous production of susceptible pigs and the introduction of immunologically naive pigs.8 On such farms, annual episodes of acute disease have been experienced. Furthermore, many herds harbor the virus without pigs having clinical signs.10,11

Reports12,13 on experimental infection of pigs with different SIV subtypes are available. However, there is little information on experimental transmission of US isolates of H1N2 virus in pigs. Experimental H1N2 virus infection in pigs with high concentrations of maternal antibodies is of particular interest because there is no information regarding protection against H1N2 virus, whereas vaccination against H1N1 and H3N2 subtypes in breeding stock is routinely practiced on commercial swine farms. The purpose of the study reported here was to evaluate clinical signs and pig-to-pig transmission when pigs with maternal antibodies to SIV subtypes H1N1, H3N2, and H1N2 were experimentally inoculated with an H1N2 or placed in contact with H1N2-inoculated pigs at different times after inoculation.

Materials and Methods

Experimental design—Ten 3-week-old pigs, seropositive for SIV subtypes H1N1, H3N2, and H1N2 antibodies, were...
purchased from a porcine reproductive and respiratory syndrome (PRRS) virus-free farm. Ten pigs (principal group) were housed in an isolation room and inoculated intranasally with SIV subtype H1N2 (A/SW/MN-1480/00; 2 mL [10^{7} TCID_{50}/mL]). Thereafter, 2 contact groups (groups 1 and 2) with 4 seropositive pigs each were placed in the room in contact with the principal pigs on days postinoculation (dpi) 7 and 28, respectively. The contact pigs remained in the room with principal pigs for 35 or 14 days and were then euthanatized. All principal and contact pigs had hemagglutination inhibition antibody titers to H1N1 (1:160 to 1:320), H3N2 (1:80 to 1:160), and H1N2 (1:20 to 1:80) at the time of inoculation or contact. Two randomly selected principal pigs each were euthanatized on dpi 4, 14, 21, 28, and 42. Four pigs each from contact groups 1 and 2 were necropsied on days postcontact (dpc) 35 and 14, respectively. The room was cleaned and examined for the presence of virus and antibody responses. Nasal swab specimens were collected from both nostrils by use of predampened swabs in tubes containing 1 mL of RPMI medium. Each swab specimen was vortexed and squeezed into a new tube. Total RNA was extracted from each specimen and used in a reverse transcriptase-polymerase chain reaction (RT-PCR) assay to detect viral RNA. At necropsy, gross lung lesions were scored via percentage of pneumatic lung volume versus total lung volume (score 1, 1% to 5%; score 2, 6% to 10%; score 3, >10%). Specimens of tonsil, trachea, lung, lymph node, heart, kidney, spleen, and blood were collected from all principal and contact pigs, and the presence of virus was examined via virus isolation and RT-PCR assay.

Serology, virus isolation, and RT-PCR assay—Sera were tested for antibodies against SIV by use of an HI technique as described. Briefly, all sera were heated at 56°C for 30 minutes and treated with a receptor-destroying enzyme to remove nonspecific inhibitors. The SIV subtypes H1N1 (A/Swine/New Jersey/11/76), H1N2 (A/SW/MN-1480/00), and H3N2 (A/Swine/Minnesota/9088-2/98) were used as reference viruses in the HI tests. Samples with HI titers ≥1:40 were considered to have positive results for antibody. Virus isolation was performed by use of Madin-Darby canine kidney (MDCK) cells. The procedure for the RT-PCR assay for the detection of viral RNA was described in detail elsewhere.

Results
Clinical, virologic, and serologic findings in principal pigs—No major clinical signs were observed in pigs after inoculation except for mild coughing and increased rectal temperatures between dpi 1 and 4 (Fig 1). Nasal swab specimens collected from all pigs on dpi 2 yielded positive results for the RT-PCR assay and the virus isolation method, and virus shedding lasted for 3 to 5 days thereafter (Table 1). Lung specimens collected from 1 of the principal pigs on dpi 42 yielded positive results via virus isolation and RT-PCR assays. There was no increase in HI antibody titer against H1N2 after virus inoculation, and gradual decreases in HI titers against H1N1, H3N2, and H1N2 were observed in sera collected on dpi 0, 7, 14, 28, and 42 from the principal group (Fig 2). No major gross lesions were observed at necropsy except that a pig

Clinical examination and sample collection—Pigs were examined for clinical signs including appetite, coughing, or behavioral changes for a period of 15 minutes daily throughout the study. Rectal temperatures were recorded for the first 7 dpi or dpc. Blood samples were collected at regular intervals and examined for the presence of virus and antibody inhibition antibody titers to H1N1 (1:160 to 1:320), H3N2 (1:80 to 1:160), and H1N2 (1:20 to 1:80) at the time of inoculation.

![Figure 1](https://example.com/figure1.png)

Figure 1—Rectal temperatures of 10 pigs (n = 8 for days 5 to 8) after intranasal inoculation (day 0) with swine influenza virus (SIV) subtype H1N2 (A/SW/MN-1480/00). All pigs had hemagglutination inhibition antibody titers to H1N1 (1:160 to 1:320), H3N2 (1:80 to 1:160), and H1N2 (1:20 to 1:80) at the time of inoculation.

Table 1—Virus detection in nasal swab specimens and lung lesion scores in 10 pigs after experimental inoculation with swine influenza virus (SIV) H1N2

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*Sign (= or –) to the left of the virgule indicates results of reverse-transcriptase polymerase chain reaction assay; sign to the right of the virgule indicates results of virus isolation. †Swine influenza virus was detected in lung at necropsy.

nt = Not tested. c = No result for virus isolation because of bacteria contamination.
Lung lesion score 0 = no lesion; 1 = 1% to 5% of lung affected; 2 = 6% to 10% of lung affected.
Clinical and virologic observations in contact pigs—Of 4 contact group-1 pigs, virus excretion was detected by use of nasal swab specimens and RT-PCR from 2 pigs on dpc 2 and from the remaining 2 pigs on dpc 6. Virus shedding detected via nasal swab specimens persisted for 4 to 9 days after the first detection. Four contact group-2 pigs were placed in the room 21 days after the introduction of the first contact group. One pig yielded positive results for viral RNA from the nasal swab specimen on dpc 4, from another pig on dpc 6, and from the remaining 2 pigs on dpc 8. Virus shedding from nasal swab specimens persisted for 2 to 6 days after first detection. No important clinical signs or lung lesions were observed in pigs of contact groups 1 and 2.

Virus distribution and identification—Trachea, lung, and lymph nodes yielded positive results for virus isolation, RT-PCR assay, or both, whereas tonsil, heart, kidney, and spleen specimens yielded negative results by use of both methods. Among 10 principal pigs, virus was detected in tracheas from 6 pigs, tonsils from 2 pigs, and a lymph node from 1 pig. Virus was not detected in the tissue specimens from 4 group-1 pigs, whereas virus was detected in the trachea of 1 group-2 pig. Results of virus isolation agreed well with those of RT-PCR assays except for 2 specimens. Virus isolation results were negative from a nasal swab specimen and a lymph node specimen, whereas RT-PCR results were positive for those specimens. All sera from principal- and contact-group pigs yielded negative results by use of virus isolation and RT-PCR assay. One isolate from each pig in principal and contact groups was tested for identity to confirm the presence of inoculated H1N2 virus. The HA genes of each isolate were amplified by use of RT-PCR and sequenced. Sequence results indicated that these isolates were identical to the inoculated virus.

Discussion

Results of this study indicated that SIV subtype H1N2 infected readily and persisted for as long as 5 days in pigs with high maternal antibody titers against H1N1 and H3N2 viruses. The virus was excreted nasally and transmitted to contact pigs housed in the same room with infected pigs. The experiment with contact pigs was designed to mimic nursery pig flow without all-in all-out management. Under a continuous production flow system, weaned pigs often move into the nursery rooms with older pigs, where they can become infected. In this study, contact group-1 pigs were infected readily from the principal pigs. Contact group-2 pigs were also readily infected, likely from the contact group-1 pigs, although 2 principal pigs and 4 contact group-1 pigs remained in the room. These results indicate that a continuous chain of infection with different subtypes of SIV could occur in nursery rooms without major clinical signs under field conditions, even in pigs with high levels of maternal immunity to SIV at weaning. Therefore, a strict all-in all-out management system with cleaning and disinfecting is highly recommended for control of SIV in pig nursery rooms.

Mild clinical signs were observed in principal pigs, whereas no clinical signs were observed in contact pigs. Duration of virus shedding was slightly shorter in contact pigs than in principal pigs. The differences in clinical signs and duration of viral shedding between the principal and contact pigs may have been caused by differences in virus infection dose. The principal pigs were infected experimentally with a high dose of SIV, whereas the contact pigs were likely infected with lower doses of excreted virus from infected pigs. Differences in the onset or duration of virus shedding have been reported after inoculation of various virus doses of 2 genetically different H3N2 SIV strains.15

After inoculation of H1N2 virus, antibody titers against H1N1, H3N2, and H1N2 viruses gradually decreased each week in all principal pigs. None of the pigs had increasing antibody titers or seroconversion against the challenge virus during our study. Decreasing antibody titers in the nursery age pigs indicated that the antibodies at the time of inoculation were of maternal origin. These maternal antibodies appeared to play an important role in protecting the pigs from clinical disease after infection with SIV.

Previous reports indicate that HI titers ≥ 1:40 are protective against clinical signs after challenge with virulent SIV subtype H1N1,16 and HI titers as low as 1:20 can inhibit seroconversion against the challenge virus.17 At the time of inoculation in our experiment, all pigs had HI antibody titers ≥ 1:20. Because of those maternal antibody titers, the pigs were not able to develop specific antibodies against the inoculated virus.

All principal and contact pigs were infected after inoculation or contact and shed virus for some period. These results suggest that high maternal HI antibody titers can reduce clinical signs but cannot provide full protection against virus infection and shedding. The maternal antibodies measured in this study were of serum origin, and it is not known whether experimental pigs had mucosal antibodies in their respiratory system. However, it is highly likely that few or no local antibodies were present at the site of infection. Humoral antibodies of maternal nature in experimen-
tal pigs may inhibit serum antibody responses but not stop local infection.

Interestingly, SIV was recovered from lung tissue of 1 principal pig 42 dpi. Such a pig can be a reservoir of virus and a source of infection in grower and finisher pigs. When the HA gene of the isolate was sequenced and compared with the original inoculum virus, we found 100% amino acid homology, although 2 nucleic acids differed. This suggests that the isolate was the same as the original inoculum virus. The 2 nucleic acid differences could be the result of nucleic acid drift, which is common among influenza viruses, or a PCR sequencing error.

Our results suggested that loss of maternal antibodies against SIV occurred during the late stage of the nursery period. This may explain why SIV outbreaks occur commonly in pigs during late nursery and early grower stages under field conditions. In the absence of piglet vaccination, SIV-related respiratory problems could occur. Therefore, a vaccination strategy along with strict all-in all-out management should be implemented in nursery rooms to control SIV infection on commercial swine farms.

References