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Experimental infection of swine influenza virus subtype H1N2 in pigs with maternal immunity

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Introduction

Since the emergence of new swine influenza virus (SIV) subtypes H3N2 and H1N2 in the U.S, respiratory disease associated with SIV has been more complicated. The H3N2 virus with genes derived from human, swine, and avian viruses has been evident since 1999. Shortly after the appearance of H3N2 virus, H1N2 virus—which resulted from reassortment between the triple reassortant H3N2 and classical H1N1 viruses—has also been isolated from pigs in at least 9 different states (Karasin et al., 2000; Choi et al., 2002).

Little information is available on experimental transmission of the US isolates of H1N2 virus in pigs with high levels of maternal antibodies, whereas SIV vaccination against H1N1 and/or H3N2 subtypes in breeding stock is routinely practiced on commercial swine farms. The goals of this study were to examine clinical signs and pig-to-pig transmission when pigs with maternal immunity were experimentally inoculated with a SIV H1N2 or placed in contact with inoculated pigs.

Experimental design

Ten 3-week-old pigs (principal group) from a PRRS-free farm were housed in an isolation room and inoculated intranasally with a SIV H1N2 (A/SW/MN-1480/00; 10^7 $TCID_{50}$/ml). All pigs had HI antibody titers to H1N1 (1:160–1:320) and H3N2 (1:80–1:160) at the time of inoculation. Two contact groups with 4 seropositive pigs each were moved into the room with the principal pigs. Contact groups 1 and 2 were placed in contact on 7dpi and 28dpi, respectively. All contact pigs had HI antibody titers to both H1N1 and H3N2 at the time of contact and remained in the room with principal pigs. Two randomly selected principal pigs each were euthanized on days 3, 7, 14, 28, and 42 post-inoculation. Pigs of the contact groups 1 and 2 were killed 33 and 10 days post-contact (dpc), respectively.

Pigs were examined for clinical signs and rectal temperatures following inoculation or contact. Nasal swabs and blood samples were collected at intervals. Total RNA was extracted from each swab and used for RT-PCR assay. Sera were tested for SIV HI antibodies, with HI titers of 1:40 considered positive. Procedure for R T-PCR assay for the detection of viral RNA were described in detail elsewhere (Choi et al., 2002). At necropsy, gross lung lesion was scored as percents of pneumonia of total lung volume (score 1=1–5%; score 2=6–10%; score 3 10%). Tissue samples were also collected and examined for virus by RT-PCR.

Results

No major clinical signs were observed in pigs following experimental inoculation with H1N2 virus except for a mild cough and elevated temperatures between 1 and 4 days post-inoculation (dpi). SIV RNA from nasal swabs was detected from all principal pigs between 1 and 12 dpi. Pigs 7 and 9 were continuously positive for viral RNA until 7 dpi. There were no increases in HI antibody titers to H1N2 following virus inoculation (Table 1), and gradual decreases in HI titers to SIV H1N1, H3N2 and H1N2 were observed in sera collected on 0, 7, 14, 28, and 42 dpi from the principal group. No major gross lesion was observed at necropsy with the exception of a pig killed at 14 dpi which showed advanced pneumonia (lung lesion score 2).

From 4 pigs in contact group 1, virus excretion in nasal swabs was detected by RT-PCR from 2 pigs on 2 dpc and from the remaining 2 pigs at 3 dpc. Virus shedding from

Table 1. Mean HI antibody titers against SIV H1N1, H3N2, and H1N2 subtypes in 10 principal pigs following inoculation with H1N2 subtype

	Mean HI antibody titers*		
days post-inoculation	H1N1	H3N2	H1N2
0	270	110	60
7	210	105	55
14	160	65	30
28	65	40	20
42	45	30	20

*SIV strains 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.

nasal swabs persisted for 4–7 days after the first detection. Four contact group 2 pigs were placed in the room 3 weeks after the introduction of the first contact group. One pig was positive for viral RNA from the nasal swab at 4 dpc. Two pigs were positive on 6 dpc, and the last pig was positive on 8 dpc. No significant clinical signs and lung lesion were observed in pigs of contact groups 1 and 2. Trachea, lung, and lymph nodes were positive for virus isolation and/or RT-PCR assays but tonsil, heart, kidney, and spleen samples were negative by both methods. All sera from principal and contact group pigs were negative by virus isolation and RT-PCR.

Discussion

Results of this study demonstrated that SIV subtype H1N2 readily infected and persisted in pigs with high HI antibody titers against H1N1 and H3N2 viruses for up to 3 weeks. The virus was excreted through nasal discharge and transmitted to contact pigs housed in the same room with infected pigs. Interestingly, SIV was recovered from lung tissue of one principal pig 42 dpi.

The HI antibody titers to H1N1 and H3N2 viruses in all of the pigs following infection gradually decreased each week, and none of the pigs showed a rising antibody titer during the experimental period. None of the pigs showed seroconversion against the challenge virus during this study. Decreasing antibody titers in the nursery age pigs indicate that the antibodies at the time of inoculation were of maternal origin. The maternal antibodies appear to play an important role in protecting offsprings from clinical disease following SIV infection.

Previous reports have indicated that HI titers of 1:40 or greater are protective against clinical signs after challenge with virulent H1N1 SIV. At the time of inoculation, all of the pigs had HI antibody titers of 1:40 or greater. Due to these high antibody titers, the pigs could not have been able to develop specific antibodies against the inoculated virus. Meanwhile, all pigs were infected following inoculation, and shed virus for some periods of time with the infection remaining in the respiratory system for up to 14 dpi. These results suggest that high maternal HI antibody titers can reduce or inhibit clinical signs but cannot provide full protection against virus infection and shedding. Furthermore, these piglets can be the reservoir of virus, thus becoming a source of infection in grower and finisher pigs. Based on our results, loss of the maternal antibodies against SIV occurred during the last stage of the nursery. This may explain why SIV outbreaks occur commonly during grow/finish stages under field conditions. In the absence of a piglet vaccine, SIV-related respiratory problems could occur. Therefore, a vaccination strategy for SIV infection should be implemented in commercial farms for grow-finish pigs.

References

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