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Challenge of pigs with natural immunity to H1 and H3 swine influenza virus with pandemic 2009 H1N1 influenza virus

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Introduction

The emergence of the pandemic 2009 human H1N1 influenza A virus raised many questions about the implications for this virus in swine (1). One such question is, does prior exposure to influenza virus confer any protection against the new virus? This report describes a study to evaluate if natural exposure to swine influenza viruses confers protection against challenge with 2009 H1N1 virus.

Materials and methods

Conventionally raised pigs were purchased from a sow herd negative for swine influenza A virus. Due to purchasing and shipping logistics, the pigs had been weaned and transported to a nursery facility that was geographically separated from the sow farm. Within 7 days of arrival at NADC most of the pigs were seropositive by the Avian Influenza MultiS-Screen ELISA (2) and virus was detected in the nasal swabs from a few pigs. Multiplex RT-PCR assays using four specific primers for H1 and H3, and N1 and N2 were performed (3). Twenty group 1 (G1) pigs were housed for 5 additional weeks (until approximately 10 weeks of age) at which time they were challenged with 2×10^6 $TCID_{50}$ A/California/04/2009 H1N1 (CA09) by the intranasal route. Naive-age-matched pigs (n=4 pigs) or group 2 (G2) were also inoculated with CA09. Nasal swabs were taken on 0, 3 and 5 days post infection (dpi) to evaluate nasal shedding. Postmortem samples including serum, bronchoalveolar lavage fluid (BALF), lung, trachea and nasal swabs were collected at necropsy at day 5 pi. Serologic assays included hemagglutination inhibition (HI) assay (4) and ELISA (2). HI assays were used to evaluate 0, 3 and 5 dpi serum samples against CA09 (H1N1), OH07 (H1N1), MN99 (H1N1), and TX98 (H3N2) viruses. Real-time PCR assays were performed for quantitation of nucleic acids in BALF of pandemic H1N1 influenza virus (5), and for PRRSV, PCV2 and Mycoplasma hyopneumoniae using in house assays (6).

Results and discussion

All pigs were negative for PRRSV, PCV2 and M. hyopneumoniae nucleic acids in BALF at necropsy. Genetic analysis of viruses isolated from nasal swabs and BALF samples from G1 pigs revealed the presence of simultaneous H1 and H3 virus infections prior to the start of the study at 5 weeks of age. After challenge with CA09, all naïve-challenged pigs (G2) had macroscopic lung lesions with an average lung involvement of 2.7%. The multifocal lesions appeared typical with well demarcated margins, purple in color. Nine of the 20 G1 pigs had detectable macroscopic lung lesions, but decreased in severity compared to the non-immune controls, with an average of 0.9%. The character of the G1 lung lesions was different compared to G2. The macroscopic lesions were purple-grey in color; involving the tips of the apical and cardiac lobes with less distinct margins. G1 pigs had no detectable virus titers in the lung or nasal swabs (0, 3 and 5 dpi). However, G2 pigs had CA09 viral titers in the lungs and nasal swabs with average titers of $10^{1.6}$ $TCID_{50}$/ml (3 dpi) and $10^{2.3}$ $TCID_{50}$/ml (5 dpi) in nasal swabs samples, and of $10^{3.7}$ $TCID_{50}$/ml BALF samples at 5 dpi. Real-time PCR for H1N1 pandemic virus in BALF (5 dpi) showed that 14/20 naturally immune pigs (G1) and 4/4 naïve-challenged (G2) were positive for viral RNA. Quantitatively G2 samples presented an average of 9×10^6copies of viral RNA / microliter of template. In contrast, G1 had an average of 1.4×10^3 copies, indicating a low viral replication in the lungs or residual RNA from challenge, confirming the negative results of virus isolation from nasal swabs and BALF. HI titers using the challenge virus (CA09) were not significant for either group at 0 and 5 dpi. However, significant HI titers against the γ-cluster SIV OH07 were detected in G1 pigs at 0 dpi. H3N2 HI antibodies against TX98 were not detected in either group of pigs. These results indicate that pigs with natural immunity against γ-cluster SIV have significant protection against infection with 2009 pandemic H1N1.

References

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