Serological Cross-Reactivity of the Novel H1N1 and Implications for Protection with a Commerical Vaccine

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Summary and Implications

The recent emergence of the pandemic H1N1 viruses and their being labeled as 'swine flu' has had several detrimental effects on the pork industry. Novel H1N1 strains have been recently detected in swine populations in the United States and in other parts of the world. While the public health significance of these findings is unknown, it is important to determine whether existing vaccines or exposure to previously circulating strains of swine influenza will protect pigs against the novel H1N1. Using a partial two-way cross-hemagglutination inhibition (HI) assay, we have determined a swine H1N1 strain present in a commercial vaccine cross-reacted with antiserum specific to the novel H1N1. We have also shown that a field serum sample with a HI high titer to a '99 H1N1 strain cross-reacted strongly with the novel H1N1. Anti-sera which were specific to the non-pandemic H1N1 strains also cross-reacted with the novel H1N1. Therefore, we conclude that current vaccines and circulating non-pandemic H1N1 field strains will provide at least partial protection against the novel H1N1 virus in pigs.

Introduction

Since April 2009, pandemic H1N1 infections have spread rapidly in human populations all over the world causing considerable morbidity and mortality. Recently a state of national emergency was declared as the number of human deaths due to the H1N1 strain exceeded one thousand. While it is not known exactly when or where the genetic re-assortment that led to the emergence of the recent pandemic H1N1 influenza occurred, it is speculated that it is the consequence of a recombination between the North American triple reassortant and Eurasian lineages of swine influenza virus (SIV). Therefore, the virus has acquired the popular name of 'swine flu' and unprecedented public health significance.

While there are no documented cases of swine-to-human transmission of the novel H1N1, recently reverse-zoonotic transmissions of the novel H1N1 from human-to-swine and poultry have been recorded. Clinical infections of the novel H1N1 in swine are indistinguishable from infections with other swine influenza strains, in terms of morbidity or mortality. Therefore, the virus may not pose a more severe threat to swine health or production when compared to commonly circulating swine influenza viruses. However, detailed experimental characterization of H1N1 pathogenesis is lacking. Surveillance for the novel H1N1 in swine and domestic birds is purely voluntary in the United States and does not require extermination in the event of discovery. However, due to the public health significance of the virus, it is important to evaluate whether existing vaccines or immunity to previously circulating SIV strains protect swine against the novel H1N1. This information is also important in making decisions regarding the need for a novel H1N1 vaccine for swine. Hemagglutination inhibition test (HI) titers have been known to correlate well with protection against influenza. In this study, we have determined the cross-reactivity of the novel H_1N_1 against selected vaccine and field strains of SIV as a first step in addressing the above question.

Materials and Methods

Virus strains and anti-sera: We obtained the novel H1N1 strain A/CA/04/2009 from the influenza branch, CDC. Specific sera against the novel H1N1 strains A/CA/04/2009 and A/ Mexico/4108/2009 were generated by infection of two pigs each. The sera samples were checked by a Multi-species Enzyme Linked Immunosorbent Assay (ELISA) which is non-subtype specific (Idexx Inc.) (Table2).

Flu Sure™ XP is a commercial swine influenza vaccine marketed by Pfizer Animal Health, Inc. (Kalamazoo, MI). It contains a human-like H1N2 virus (Pfizer 031 – 95% identical to A/Hu/NY/294/2003), a H1N1 virus isolated in 1999 (Pfizer 161 – 95% identical to A/SW/IN/2000) and a cluster IV H3N2 virus. The human-like H1N1 and the reassortant H1N1 vaccine virus strains were provided by Pfizer Inc. for routine testing of client samples in the ISU-VDL serology section. Antisera specific to the Pfizer human-like H1N1 and the reassortant H1N1 vaccine strains were also provided by Pfizer Inc. A classical H1N1 virus isolated in 1973 and a H1N1 strain (35233-99) isolated in 1999 were available to us. A field sample with a known high titer to the 35233-99 strain was used to determine cross-reactivity to the novel H1N1. Swine sera samples generated from a previously published study where eight groups of four pigs each were infected with swine influenza viruses, which are representative of the three currently circulating swine influenza virus subtypes H1N1, H1N2 and H3N2 (Table 2), were provided by Dr. Bruce Janke, ISU-VDL.

Culture of the novel H1N1 virus strain A/CA/04/2009: MDCK cells were cultured for 48-72 hrs until an approximate ~80% monolayer was achieved. Monolayered cells were inoculated with A/CA/04/2009 virus. Cells incubated for 2 hours in a 5%, 37°C, CO_2 incubator. The virus was removed from the cells and new medium with 10% fetal bovine serum (FBS) was added to the cells. Cells were incubated for 36-48 hours until 25-75 % cytopathic effect (CPE) was observed. Thereafter, flasks were frozen to below -70°C and thawed to release cell-associated virus. Cultures were centrifuged and tested for HA units before aliquoting and storing virus for future use.

Hemagglutination inhibition assay: 50 µl of each serum sample and high positive, low positive and negative controls were added to a deep well plate. Samples were pretreated with heat-inactivation, 20% Kaolin, and 0.4% rooster red blood cells (RBC) before transferring to dilution plate. Serial two-fold dilutions were performed on samples in a dilution plate with one row serving as a negative control. 8 HA units of the virus were added to the diluted serum on a separate micro-titer plate and incubated for 40 minutes. 0.4% rooster RBC solution was added and incubated for 60 minutes. Plates were assessed for the formation of a button or crenation.

Results and Discussion

Pigs infected with the A/ Mexico/4108/2009 novel H1N1 strain appeared to mount lower homologous hemagglutinating antibody responses compared to pigs infected with the A/CA/04/2009 strain. Both novel H1N1 strains cross-reacted with the 35233-99 Iowa '99 H1N1 strain, while A/CA/04/2009 cross-reacted with the 1973 classical H1N1 strain and the A/ Mexico/4108/2009 strain did not. However, this difference could be attributed to the low homologous HI titers for this virus.

Importantly, the A/Mexico/4108/2009 and A/CA/04/2009 specific antisera cross-reacted with the Pfizer re-assortant H1N1 vaccine strain although the titers were not high, indicating that currently available vaccine would confer a degree of protection against the novel H1N1. In confirmation, antisera raised against the two vaccine virus strains described in Table 1, cross-reacted with A/CA/04/2009. However, negligible there was negligible cross-reactivity between the human-like H1N1 vaccine strain and A/CA/04/2009 (Table 1).

More interestingly, a field serum sample which was found to have high HI titers to the 35233-99 Iowa '99 H1N1 strain reacted equally as strong with the novel H1N1 strains, indicating that a pre-existing and robust protection against the novel H1N1 strains is highly likely in field conditions. However, this field serum sample is not very well characterized in terms of the other viruses the pig might have been exposed to.

Similarly, A/CA/04/2009 showed varying levels of HI activity with pigs experimentally infected with the classical H1N1, re-assortant H1N1, and two clusters of H1N2 (Table 2). As expected, A/CA/04/2009 showed no cross-reactivity with serum from pigs experimentally infected with three different H3N2 clusters.

Therefore, we speculate that existing SIV vaccines will confer a degree of protection against the novel H1N1 and that currently circulating SIV strains can serve the same function. However, as the novel H1N1 has been recently transmitted from humans to swine in different parts of the world, it is important to scientifically assess whether such existing protection can provide herd-level immunity. It is also important to recognize that sub-optimal levels of protection in a population could result in negative selection pressure which may force the virus to evolve into more pathogenic forms, especially given the public health significance of the novel H1N1. More systematic and thorough studies are required to address this critical question.

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Table 1. Antigenic cross-reactivity of the novel H1N1 with vaccine strains.

Serum/ Virus	Novel H1N1 (A/CA/04 /2009) *	35233-99 Iowa '99 H1N1*	Pfizer Re-assortant H1N1*	Pfizer H031 (Human-like H1N2)*	1973 classical H1N1 (-ISU-VDL)*
A/CA/04/2009 Pig 574	160	160	40	≤ 10	20
A/CA/04/2009 Pig 575	320	80	40	≤ 10	40
A/ Mexico/4108/09 Pig 603	80	20	20	≤ 10	≤ 10
A/ Mexico/4108/09 Pig 604	40	40	20	≤ 10	≤ 10
Field sample with high H1N1 titer	**640**	640			
Pfizer re-assortant H1N1 Positive control	**40**	640	640	≤ 10	
Pfizer H031 Human-like H1N2	**20**			640	
Negative controls	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10

* Mean reciprocal HI titer at 8 HA units of each virus

Table 2. Cross- reactivity of the novel H1N1 virus to previously circulating SIV strains.

Anti-sera	Mean reciprocal HI titer against H1N1 virus A/CA/04/2009 +,$	Mean SIV titer *,$
cH1N1(A/Swine/IA/40776/92)	33	0.09
tH1N1A(A/Swine/H02NJ56371/02 +H3N2)	40	0.2
tH1N1B(A/Swine/IA/35233/99)	**≤ 10**	0.32
H1N2A(A/Swine/Wisconsin/R33f/01)	42.5	0.15
H1N2B(A/Swine/IA/35233/99)	13.33	0.18
H3N2C1(A/Swine/Texas/4199-2/98)	≤ 10	0.1
H3N2C2(A/Swine/Texas/4199-2/98)	≤ 10	0.33
H3N2C3(A/Swine/Texas/00036/02)	≤ 10	0.25
Pandemic H1N1 (A/CA/04/2009)	135	0.11
Negative control	≤ 10	0.92

***** -samples with S: N ratios above 0.5 are considered negative
+ mean reciprocal HI titer with 8 HA units of each virus
$ mean values for four pigs in each group