INTERNSHIP ARTICLE

Swine Origin A/Sw (H3N2) Influenza Virus is Transmissible Through Air in Guinea Pigs

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Abstract

Recent isolation of a novel swine-origin influenza A H3N2 variant virus [A(H3N2)v] from humans in the United States has raised concern over the pandemic potential of these viruses. The goal of this study was to compare the mammalian transmissibility of an A/Sw (H3N2) swine virus, which was isolated from a pig, to a genetically similar A(H3N2)v virus, isolated from a human, to determine if the human isolate has increased transmission potential. Four Guinea Pigs were inoculated with A/Sw (H3N2) virus and 4 were left naive. The Guinea Pigs were placed in separate aerosol cages. Nasal washes were performed every two days for ten days to test for traces of infection in naive Guinea Pigs. If influenza A/Sw (H3N2) virus is transmissible through the air, then it will infect the naïve Guinea Pigs exposed to the infected animals. The results at the end of the experiment suggest that the influenza A/Sw (H3N2) virus is indeed transmissible through air, similar to the results previously obtained with the human A(H3N2)v virus.

Introduction

Seasonal epidemics are an international public health burden. During seasonal epidemics, the risk for hospitalization and death are highest among all ages. The risk for hospitalization and/or death from influenza during flu season is actually highest for the very young, less than 2 years, and the very old, more than 65 years.

Although influenza viruses adapted to and circulating among pigs have been known to infect humans in the past, a new swine virus, called influenza A(H3N2), variant [A(H3N2)v] virus was isolated from two children in the summer of 2011. This variant virus was different from prior swine A(H3N2) viruses in that it was a swine-human reassortant, encoding the M gene segment from the 2009 pandemic H1N1 virus that circulated among humans, in a swine A(H3N2) background[1]. In the summer of 2012, a total of 153 human cases of A(H3N2)v infection were reported, compared to 29 cases the prior year. Although the vast majority of cases had exposure to pigs, usually in the setting of livestock shows at county fairs, the increase in swine-to-human transmission has sparked a concern that the A(H3N2)v virus may be more transmissible from human-to-human than prior swine A(H3N2) [A/Sw (H3N2)] viruses[2]. If the influenza A (H3N2)v virus is transmissible through air in an experimental animal model, then it might indicate that it could also spread easily and rapidly among humans, possibly affecting a large population. Because these special "variant" viruses (with the pandemic H1N1 "M" gene) are increasingly being isolated from humans, the concern is that they are becoming better adapted to humans and thus might become transmissible from human to human - which could cause a pandemic.

In a previous experiment, it was confirmed that the [A(H3N2)v] virus has the capacity for efficient replication and transmission in mammals[3]. However, in this experiment, we will be testing if the human virus transmits better than the swine virus, in guinea pigs, as we already know that the swine virus does not transmit well among humans. Thus, the A/Sw (H3N2) virus was inoculated into 4 guinea pigs. Each guinea pig was then placed with a naive guinea pig. However, they were in separate aerosol cages; therefore they did not come into physical contact with each other. The cages were placed inside an air circulator. The results show that the A/Sw (H3N2) virus is transmissible through the air in guinea pigs, as naive guinea pigs became infected with the virus.

Materials and Methods

Viruses and Cells: The virus tested in these experiments, influenza A/Swine/Indiana/27-1122/2010(H3N2) [A/Sw(H3N2)], was isolated from a pig in Indiana in 2010. The guinea pig transmission of this virus will be compared to that of a human variant virus, A/Indiana/08/2011(H3N2)v, which was performed previously. Both viruses were the generous gift of Dr. Richard Webby, St. Jude's Children's Research Hospital, Memphis TN. Madin-Darby canine kidney (MDCK) cells were maintained in minimum essential medium (MEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, and 100 units/ml of penicillin plus 100 µg/ml of streptomycin (1x P/S). Virus infections were performed in a low-serum medium consisting of Opti-MEM (Gibco) supplemented with 0.3% bovine serum albumin (BSA), 0.1% FBS, 1x P/S, and 1 µg/ml of TPCK-treated trypsin (Sigma-Aldrich). The original virus stock (128 hemagglutination units (HAU)) was diluted 1:200 in PBS supplemented with 0.3% bovine serum albumin and 1x P/S and filtered through a 22 µm filter (Millipore SteriFlip). Virus was inoculated onto MDCK cells (2 x10^7 cells) and allowed to grow in low-serum medium for 4 days at 33° C and 5% CO_2. *Animals:* Five- to six-week-old female Hartley strain guinea pigs were obtained from Charles River Laboratories (Kingston, NY). Animals were allowed access to food and water ad libitum and kept on a 12-hour light-dark cycle. This study was carried out in strict accordance with recommendations in the Guide for the Care and Use of Laboratory Animals. *Transmission Experiments:* Transmission experiments were performed in an aerosol/droplet

model as previously described[4,5,6] in an environmentally controlled chamber (Caron model 6030) set at 20° C and 20% relative humidity. On Day 0, all 8 guinea pigs were anesthetized with ketamine (30 mg/kg) and xylazine (5 mg/kg) given intramuscularly. All animals had blood drawn by lateral saphenous vein puncture. Briefly, the fur from the ankle was shaved off to prevent the blood from getting caught in the fur, and a thin film of petroleum jelly was spread over the skin. The Guinea Pigs had an alcohol pad rubbed over the area to make the lateral saphenous vein more visible. The saphenous vein was punctured with a 26-gauge needle, and blood welling up on the petroleum jelly was aspirated up by pipette. Finally, 4 donor guinea pigs were inoculated with 104 plaque-forming units (pfu) of virus suspended in 300 µl of PBS+BSA supplemented with 1x P/S (PBS+BSA+P/S), instilled intranasally. On day 1 post-inoculation (pi), one naïve guinea pig was paired with one inoculated guinea pig, with each animal placed into a standard polypropylene rodent cage that has been modified by replacing one plastic side wall with a metal grille, as previously described[6]. The two paired cages were positioned with wire sides opposed, so that air can circulate freely between the cages of inoculated and exposed guinea pigs. Exposed animals were handled before inoculated animals, with gloves changed and work surfaces sanitized between animals. *Quantification of Viral Titers:* Guinea pigs were weighed every two days between 2 and 10 days post-inoculation (dpi). By keeping track of their weight throughout the experiment, we were able to see how the Guinea Pigs were affected by the influenza A (H3N2) virus, if they were infected. Nasal wash samples were collected from inoculated and exposed animals at 2, 4, 6, and 8 dpi and from exposed animals only at 10 dpi. Nasal washing was performed as previously described[4]. Briefly, nasal washing was performed by instilling a total of 1 ml of PBS-BSA-P/S into the nostrils and allowing it to drain onto a sterile petri dish. Samples were collected in 1.5-ml tubes, centrifuged to pellet debris, and stored at -80°C before analysis. Nasal wash virus titers were determined on confluent monolayers of MDCK cells by plaque assay of thawed nasal wash samples, serially diluted from 1:2 to 1:105. Plaques were visualized by immunostaining[7] with A/Indiana/08/2011(H3N2)v-infected guinea pig serum as a primary antibody. Nasal wash titers (in log10 pfu/ml) are graphed as a function of time. *Hemagglutination Inhibition (HI) Assay:* Guinea pig serum collected before and after infection was assessed for the presence of specific anti-influenza virus antibodies by HI assay. Serum was treated by trypsin-heat-periodate to inactivate any non-specific inhibitors of hemagglutination in the guinea pig serum. A half volume of trypsin solution was added to 1 volume of serum. This solution was heated at 56° C for 30 minutes and later on cooled to room temperature. Three volumes of 0.011 M metapotassium periodate (KIO_4) was added to the solution. The solution was mixed and left at room temperature for 15 minutes. Three volume of 1% glycerol saline was added to the solution, and again mixed and left at room temperature for 15 minutes. Two and a half volumes of 0.85% saline was added to the solution and mixed. The final serum dilution after treatment was 1:10. Inactivated serum was then diluted 2-fold across a 96-well microplate, from 1:20 to 1:2560 (8 wells) and then incubated with A/Sw(H3N2) virus, to allow influenza virus-specific antibodies in the serum to bind to the virus. Then an equivalent volume of turkey red blood cells (RBCs), diluted to a hematocrit of 0.5%, was added to each well, and the mixtures were allowed to settle. In the presence of influenza virus-specific antibodies, the A/Sw(H3N2) virus would be unable to hemagglutinate the turkey RBCs, and they would fall to the bottom of the well, forming a pellet. In the absence of influenza virus-specific antibodies, the A/Sw(H3N2) virus would bind to hemagglutinin receptors on the RBCs and retain RBCs in solution.

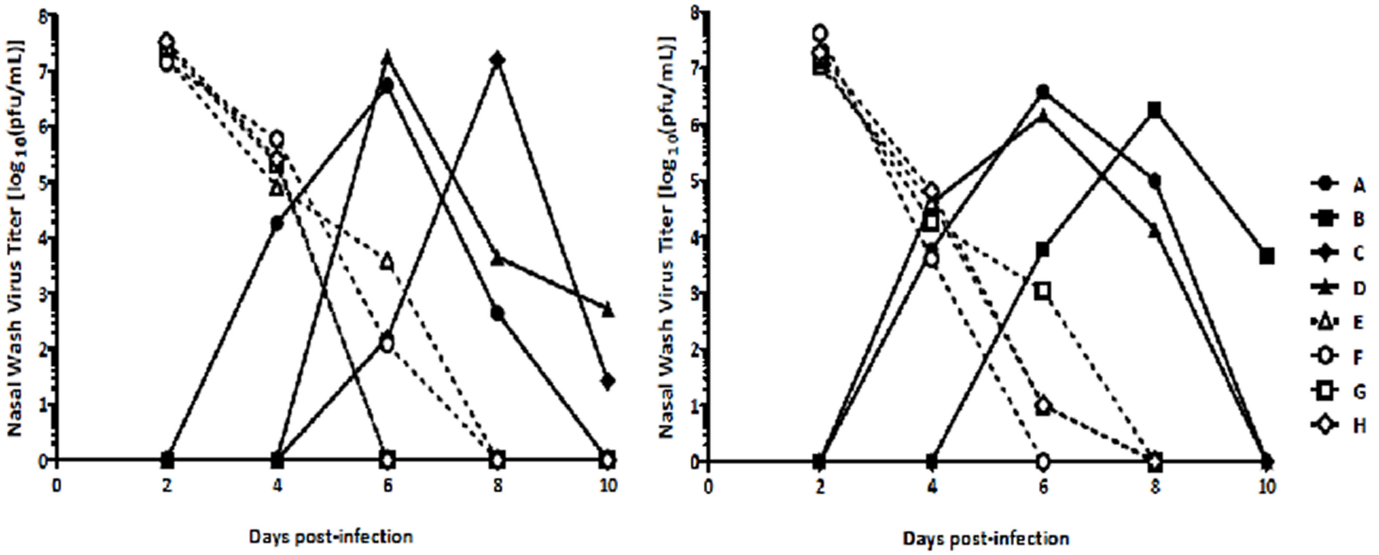

Figure 1. Nasal wash virus titers as a function of time post-inoculation. *(Left panel)* Nasal wash virus titers from guinea pigs inoculated with A/Swine/Indiana/27-1122/2010(H3N2) virus are represented by dotted lines, while those that became infected by airborne transmission from an inoculated animal are represented by solid lines. The nasal virus titers of both inoculated and transmission-infected guinea pigs were very similar to titers obtained from guinea pigs infected with the human isolate A/Indiana/08/2011(H3N2)v, performed previously *(right panel)*. The left panel shows only the 3 guinea pigs which became infected.

Results

After 10 days of inoculation 3 of the 4 exposed guinea pigs were infected with the 27-1122 virus as well as all of the inoculated guinea pigs. The virus titers for the naive guinea pigs were approximately the same as for the human variant virus that was tested previously (A/IN/08/2011(H3N2)v) (Figure 1). 1 of the 4 guinea pigs remained virus-free after the 10 days of inoculation.

To confirm the nasal wash results, blood was drawn from all 4 exposed guinea pigs 2 weeks after the end of the transmission experiment. These post-infection serum samples were then tested for the development of influenza virus-specific antibodies. Three of the 4 exposed guinea pigs demonstrated a greater than 4-fold rise in influenza virus-specific antibodies by hemagglutination inhibition (HI) assay (Table 1), confirming the nasal wash results. Only guinea pig B failed to develop influenza virus-specific antibodies; guinea pig B was also the only one in whose nasal washes the test virus A/Sw(H3N2) could not be detected (Figure 1 left panel).

	Antibody Titer by HI Assay		
Guinea Pig	Pre-infection	Post-infection	Fold increase in antibody titer
A	1:40	1:2560	64
B	1:40	<1:40	None
C	1:80	1:320	4
D	1:40	1:1280	32

Table 1. Seroconversion in virus-naïve guinea pigs exposed to A/Sw(H3N2)-infected guinea pigs.

Discussion

This study examined if the A (H3N2) virus was easily transmitted through the air. Since its discovery in swine in 2010, the influenza A(H3N2)v virus has been increasingly isolated from humans, most but not all of whom had some contact with livestock pigs. Because previous swine A(H3N2) viruses, such as those that had not yet acquired the M gene segment from the human pandemic 2009 A(H1N1) virus, were much more rarely able to make the swine-to-human transition, we wondered whether the A(H3N2)v virus was better adapted to humans and thus might transmit more readily among them.

Due to our results, we believed that the Influenza A (H3N2) virus is easily transmissible through air. After observing 4 originally naïve Guinea Pigs and 4 inoculated Guinea Pigs, we found that 3 out of the 4 originally naïve Guinea Pigs became infected with the A/Sw(H3N2) virus while being in an environmentally controlled chamber for a period of 10 days. The timing and frequency of transmission of the A/Sw(H3N2) virus was similar to that of a previously tested human A(H3N2)v isolate; therefore, it does not appear that the A(H3N2)v virus is able to transmit more efficiently than the swine virus in this animal model. However, these experiments will be repeated to ensure that these results are reproducible in independent replicates.

Sources of experimental error include accidental infection of a naïve guinea pig by experimenters, instead of by inhaling virus particles exhaled by its infected partner guinea pig. Also, the Hartley guinea pigs used in these experiments are outbred, which means that they are not genetically identical, as are many experimental mouse strains. Other errors in this experiment include errors in our cell culture, materials, equipment, environment, cells, and technique.

However, it is important to note that the A/Indiana/08/2011(H3N2)v virus was isolated in the summer of 2011, when only 29 human cases were identified. In the summer of 2012, 153 human infections were documented, so it is possible that the A(H3N2)v variant virus subtype has undergone further evolutionary changes since this particular virus specimen was isolated. It may be necessary also to test a virus isolated in 2012, to see whether it demonstrates enhanced transmissibility in the guinea pig model. Regardless, these data indicate that, even though swine-origin variant H3N2 influenza viruses seem to be more capable of infecting humans, they may not be more capable of transmitting from person to person, thus initiating a human pandemic.

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