RESEARCHREPORT
SWINE HEALTH

Title: Influenza Vaccination of Pigs in the Presence of Maternally-Derived Antibodies - **NPB #08-050 revised**

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Industry Summary

A vaccine was constructed using a gene from swine influenza and structural genes from alphavirus as the vaccine backbone. This vaccine induced both immunity and strong protection when given to pigs with no antibodies to influenza derived by suckling. Given the history of influenza viruses to change quickly, this vector system is well suited for influenza vaccine generation as one can rapidly produce vaccines with this method. However, if pigs did receive antibodies to influenza by suckling, the vaccine was unable to overcome the maternal antibodies and did not induce immunity and protection. Possibly, producers could avoid maternal antibody interference by vaccinating sows and gilts with currently available vaccines and then giving pigs vaccines not yet developed and readily available that is based on flu proteins that are not affected by maternal antibody.

Keywords: swine influenza virus, replicon particle vaccine, hemagglutinin, swine, maternal antibody

Scientific Abstract

An alphavirus derived replicon particle (RP) vaccine expressing the cluster IV H3N2 swine influenza virus (SIV) hemagglutinin (HA) gene induced protective immunity against homologous influenza virus challenge. Vaccination of immunologic naive pigs (no maternal antibody to SIV) induced antibodies to the HA protein. In subsequent experimental challenge with a homologous H3N2 strain, the vaccine prevented an increase in temperature, reduced gross and histologic lesions in the lungs, and reduced viral replication. However, pigs with maternal antibody were not protected against challenge when vaccinated with RP vaccines expressing HA alone or in combination with nucleoprotein.

Introduction

Swine influenza virus (SIV) is cause for serious concern in the swine industry due to clinical sequelae, delayed time to market, and zoonotic potential. Pigs have been implicated in transmission of influenza to humans. Continuous mutations of the hemagglutinin (HA) and neuraminidase (NA) genes lead to antigenic differences; contributing to vaccine ineffectiveness, epidemics and/or pandemics. Protective immunity is primarily due to antibodies against HA but antibodies to other gene products, such as NA, and cell-mediated immunity to

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nucleocapsid protein (NP) may play a role as well. Existing commercial SIV vaccines are effective when given to swine without maternal antibodies if the vaccine contains the antigenically related virus present in subsequent exposure. The objectives of this study were to determine if 1) a replicon particle (RP) vaccine derived from an alphavirus and expressing the HA gene could induce immunity and protection to homologous SIV in the absence of maternal antibody and 2) RP vaccines expressing influenza genes could induce an immune response and protection in the presence of maternal antibody.

Objectives

Swine influenza virus (SIV) is a serious disease in the swine industry due to clinical disease, delayed time to market, and zoonotic potential. A major hurdle for SIV vaccination is maternally-derived antibodies. This passive immunity is beneficial for protecting newborn piglets from clinical disease but detrimental to the induction of active immunity via vaccines and the desired disease protection. Overcoming this obstacle would be very helpful to swine producers. We have preliminary evidence that replicon particle recombinant (RP) vaccine expressing hemagglutinin induces hemagglutinating-inhibiting (HI) antibody against SIV in the presence of maternally-derived antibody and propose to verify and extend these results. We also propose to determine if replicon particles (RP) vaccine protects young pigs having maternally-derived antibody against challenge with SIV.

Materials and Methods

The virulent cluster IV H3N2 SIV was isolated from a clinical case of influenza in a pig from Iowa in 2007 and amplified on Madin-Darby canine kidney cells (MDCK). This virus was the source of the HA gene used in the RP vaccine. It was used to create a homologous hemagglutinatin inhibition (HI) assay, the virus source for individual pig challenge and was contained in the commercial SIV vaccine.

To construct the RP vaccine, replicon plasmids containing the HA derived from the SIV isolate or the nucleocapsid (NP) gene derived from the human influenza virus isolate A/Wyoming/03/2003 (H3N2) and helper plasmids containing alphavirus Venezuelan equine encephalomyelitis (VEE) capsid and glycoprotein genes were prepared and titter determined using a modification of previously described procedures. Each gene was then cloned into a replicon vector using the unique EcoRV and AscI restriction sites. The replicon and helper RNAs were mixed with Vero cells in electroporation chambers and pulsed. Replicon electroporated cells were incubated at 37°C for 18 hours. RPs were tested in a cytopathic effect assay to assure the absence of detectable replication-competent virus.

In experiment 1, pigs came from two sources. One source was SIV antibody negative and did not use SIV vaccine; the other source routinely used commercial SIV vaccine in gilts and sows. This resulted in pigs with no maternal antibody (Groups 1 and 2) or maternal antibody to SIV (Groups 3 – 5). Piglets were weaned at 2 ½ weeks of age, transported to Iowa State University livestock infectious disease isolation facility and randomly divided into 5 groups of 10 pigs each. Pigs were immunized at 3 and 5 weeks of age (Days 0 and 14, respectively). Pigs either received 2 ml of phosphate buffered saline (PBS) containing 5.6×10^8 RP expressing the HA gene, commercial vaccine or a placebo containing only PBS intramuscularly (Experiment 1).

In Experiment 2, piglets were weaned at 2 ½ weeks of age, transported to Iowa State University livestock infectious disease isolation facility and randomly divided into 3 groups of 10 pigs each. Pigs were immunized at 3 and 5 weeks of age (Days 0 and 14, respectively). All pigs in Experiment 2 had passively acquired maternal antibody and were vaccinated with either 2 ml of PBS containing 1.3×10^9 HA RP and 2.6×10^7 NP RP, 2 ml of PBS containing 1.3×10^9 HA RP and 2.6×10^7 NP RP day 0 prime followed by 2 ml boost with commercial vaccine day 14, or a placebo containing only PBS intramuscularly. All pigs in both experiments were challenged intratracheally with 10 ml containing 1×10^7 $TCID_{50}$ (50% tissue culture infectious dose) SIV. This time was chosen because maternally acquired passive antibodies to HA were estimated to have decreased

to a point as to not be inhibitory to the virus challenge. The experimental design for both experiments is summarized in Table 1.

The HI assay that measures antibodies to HA protein was performed on sera. Briefly, sera collected from pigs were treated overnight with receptor destroying enzyme (Kaolin, Sigma Aldrich, St. Loius, MO), heat inactivated, and diluted 1:9 in sterile PBS (pH 7.4) to be used in the homologous HI assay. The HI assay was performed using 0.4% turkey erythrocytes and four hemagglutinating units of homologous cluster IV H3N2 SIV.

Nasal swabs collected at 4 days post-challenge, and bronchioalveolar lavage (BAL), collected at necropsy (4 days post challenge), were quantitatively evaluated for the presence of viral RNA using real-time reverse transcription PCR (RT-PCR). Briefly, nasal samples were collected using sterile swabs to scrape the dorsal naso-pharyngeal area. Swabs were then placed in tubes containing 1 ml sterile Dulbelco's minimal essential medium (DMEM) with antibiotic (100 µg/ml gentamicin) and stored at -80˚C until assayed. The BAL was performed by pipetting 50 ml of sterile DMEM with antibiotic into the lungs of euthanized animals and extracting back into sterile conical tubes.

Results

Experiment 1

In pigs with no maternal antibody, the HA RP vaccine induced an antibody response to the HA antigen when compared to the placebo (Fig. 1; Groups 1 and 2). HA RP vaccinated pigs demonstrated a geometric mean homologous HI titer of 367 (range 160-1280) on the day of challenge (day 53). Placebo vaccinated animals had no detectable antibody to the HA antigen at day 14 or any other subsequent time. In experiment one, all three groups with maternal antibodies had high homologous HI titers at day 0 that decreased over time and were at non-protective levels (≤ 20) at the day of challenge (day 53; Fig. 1; Groups 3 - 5).

No difference in mean rectal temperatures was noted 1 day after vaccination with HA RP, commercial vaccine or placebo when compared to mean temperatures the day of vaccination (Figure 2; day 0 vs. day 1 and day 14 vs. day 15). In the two groups with no maternal antibody, the placebo group without maternal antibody (Group 1) significantly increased from day of challenge to 1 day PC (mean ± std; 39.8 ± 0.3 to 40.8 ± 0.8). The group with no maternal antibody given HA RP vaccine did not have a significant mean temperature increase from day of challenge to 1 day PC. The temperature increase from day of challenge to 1 day PC was seen in the maternal antibody group given 2 doses of RP HA vaccine (mean ± std ; 39.8 ± 0.4 to 40.7 ± 0.7) but not in the placebo or commercial vaccine group. The mean rectal temperature of all five groups in Experiment 1, regardless of presence or absence of maternal antibody, returned to day of challenge temperature on 2 and 3 days PC.

There was significantly more viral RNA detected in nasal swabs and BAL 4 day PC (day 57) in placebo vaccinated, maternal antibody negative pigs, 4.1 $\log_{10}$/ml and 4.6 $\log_{10}$/ml respectively (Figure 3; Group 1), than in HA vaccinated maternal antibody negative pigs, 0.5 $\log_{10}$/ml and 2.8 $\log_{10}$/ml respectively (Figure 3; Group 2). The amount of viral RNA in nasal swabs in all three groups with maternal antibody (HA RP, commercial, or placebo) was not significantly different (Figure 3; Groups 3-5). However, group 5 given commercial vaccine did have significantly less viral RNA in BAL than in the placebo or HA RP group (Figure 3; Groups 3-5).

The gross lung lesions and histologic lesions in pigs without maternal antibody vaccinated with HA RP (7.4 and 0.1 percent, respectively) was significantly lower than in pigs without maternal antibody given placebo and the three groups with maternal antibody (Table 2).

Experiment 2

All groups, including the vaccinated groups, had antibodies on day 0 that decreased steadily over time to non-protective levels (≤ 20) by the day of challenge. The temperature increase from day 1 PC compared to day of challenge was not seen in the group receiving 2 doses of the HA and NP RP vaccine but the temperature increase on day 1 was noted in the other two groups (group 1 mean ± std, 39.8 ± 0.3 to 40.7 ± 0.6; group 3 mean ± std, 39.7 ± 0.3 to 40.8 ± 0.7). The amount of viral RNA in nasal swabs and BAL in pigs with maternally-derived antibody was similar in all three groups (Figure 4). The gross and histological lung lesions of the group given two doses of HA and NP RP vaccine were not different than the group given placebo or the group primed with HA and NP RP and boosted with commercial vaccine (Table 3).

Discussion

This work is the first demonstration of the use of RP vaccine to induce protection against challenge with cluster IV H3N2 isolated from swine. The RP system can be used as a vaccine for influenza virus isolated from swine. The HA RP vaccine induced a strong antibody response (homologous HI assay) prior to virus challenge and imparted the following after virus challenge: 1) reduced viral RNA in nasal swabs and BAL samples 2) prevention of a significant febrile response and 3) reduced gross and histological lung damage. Given the history of influenza viruses to change quickly, this vector system is well suited for influenza vaccine generation.

The RP vaccine induced immunity that was very similar to that achieved by others using commercial vaccine. Erdman et al previously reported that RP vaccine derived from alphavirus and the HA gene from human influenza virus isolate A/Wyoming/03/2003 (H3N2) could induce high levels of HI antibody in swine but this study did not involve virus challenge. Vander Veen et al recently showed that vaccination with an alphavirus replicon expressed recombinant HA protein protects pigs against challenge with the virus A/California/04/2009. An advantage to using RP vaccines is that parental vaccination leads to both systemic and mucosal immunity, but the exact mechanism is unknown.

We were unable to overcome maternal antibody inhibition despite 4 different vaccination protocols (experiments 1 and 2). Wesley et al used an adenovirus vector expressing swine HA and NP to prime the immune system and then boosted with a commercial SIV to induce HI titers even though pigs had maternal antibody at the time of vaccination. We used a similar approach in experiment 2 but were not successful in overcoming the inhibitory effects of maternal antibody. The RP vaccine NP gene used in the current study was only 89 percent homologous with the challenge SIV and that may partially explain our inability to overcome maternal antibody interference.

Infection of young pigs with maternal antibody results in an immune response and since RP does replicate once, but not propagate, we hoped that its use as a vaccine would result in immunity despite the presence of maternal antibodies. Possibly, one could vaccinate the sows with conventional vaccine that gives strong protection based on antibodies to HA. While these antibodies do blunt the immune response to conventional vaccines in younger pigs, one could use a vaccine in younger pigs that contains flu proteins other than HA. The NP protein and the extracellular portion of matrix 2 protein are good candidates for vaccination of young pigs and our preliminary evidence suggests one can reduce infection with flu via vaccination using this method.

Table 1
Experimental design. Pigs in all groups were challenged on Day 53 with swine influenza virus H3N2 and necropsied on Day 57

	Group	No. Pigs	Maternal antibody	Vaccine Type First dose (Day 0)	Vaccine Type Second dose (Day 14)
Exp. 1	1	10	Neg	PBS[1]	PBS
	2	10	Neg	HA RP [2]	HA RP
	3	10	Pos	PBS	PBS
	4	10	Pos	HA RP	HA RP
	5	10	Pos	Commercial[3]	Commercial
Exp. 2	1	10	Pos	PBS	PBS
	2	10	Pos	HA RP + NP RP[4]	HA RP + NP RP
	3	10	Pos	HA RP + NP RP	Commercial

[1] Phosphate buffered saline
[2] Hemagglutinin gene in replicon particle
[3] Commercial H3N2 vaccine (Newport Laboratories, 1520 Prairie Drive, Worthington, MN 56187)
[4] Hemagglutinin and nucleoprotein genes in replicon particles

Table 2
Experiment 1. Gross lung lesions (mean percents and ranges) and histological lung scores (means and ranges) at necropsy (Day 57)

Group		Gross	Histologic
1	mean	20.8[b]	1.1[b]
	range	3 to 35	0 to 2
2	mean	7.4[a]	0.1[a]
	range	2 to 15	0 to 1
3	mean	12.4[b]	0.7[b]
	range	1 to 35	0 to 2
4	mean	21.9[b]	1.2[b]
	range	5 to 37	0 to 2
5	mean	9.2[b]	0.2[b]
	range	1 to 48	0 to 2

[a,b] Groups with different letter superscripts are significantly different from each other at $P < 0.05$

Table 3
Experiment 2. Gross lung lesions (mean percents and ranges) and histological lung scores (means and ranges) at necropsy (Day 57)

Group		Gross	Histologic
1	mean	15.1	1.1
	range	4 to 19	0 to 2
2	mean	21.6	1.6
	range	9 to 39	0 to 3
3	mean	25.9	1.3
	range	5 to 47	0 to 2

There were no significant differences between the groups

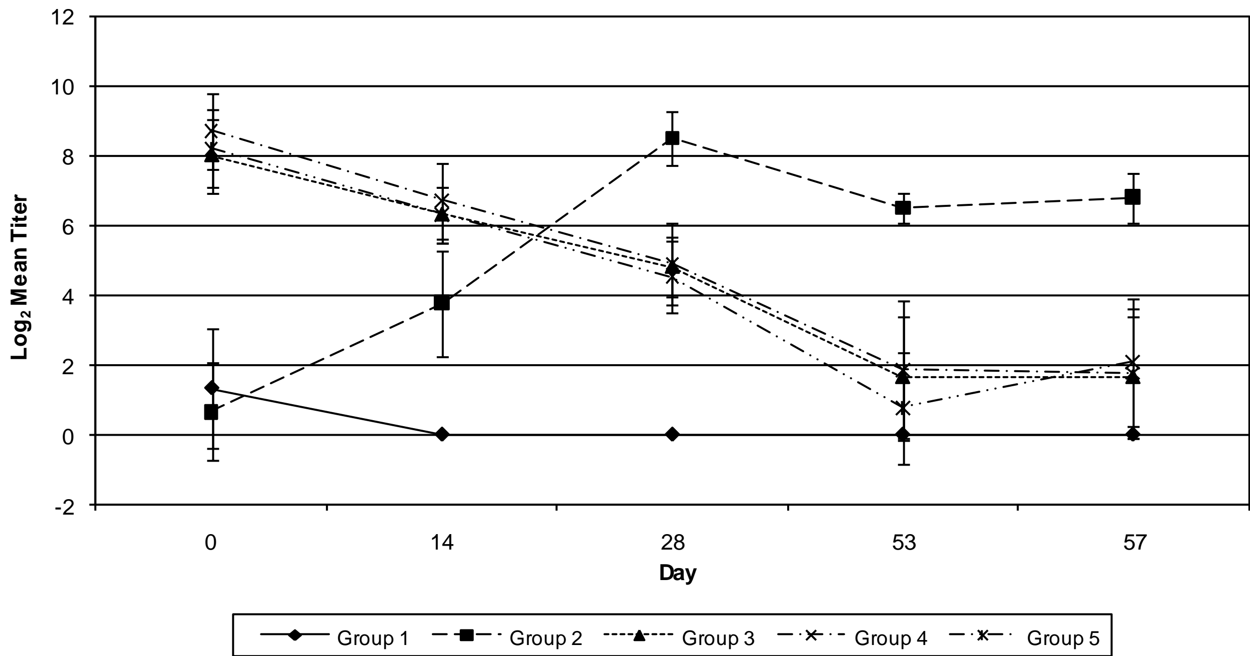

Fig. 1. Experiment 1. Hemagglutination inhibition test results. Mean titers ($\log_2$) ± std for each group at 5 sampling times. Groups 1 and 2 had no maternal antibody while groups 3, 4, and 5 did. Groups 2 and 4 were vaccinated with HA RP at day 0 and 14, groups 1 and 3 were vaccinated with placebo at day 0 and 14, and group 5 was given commercial vaccine at day 0 and 14.

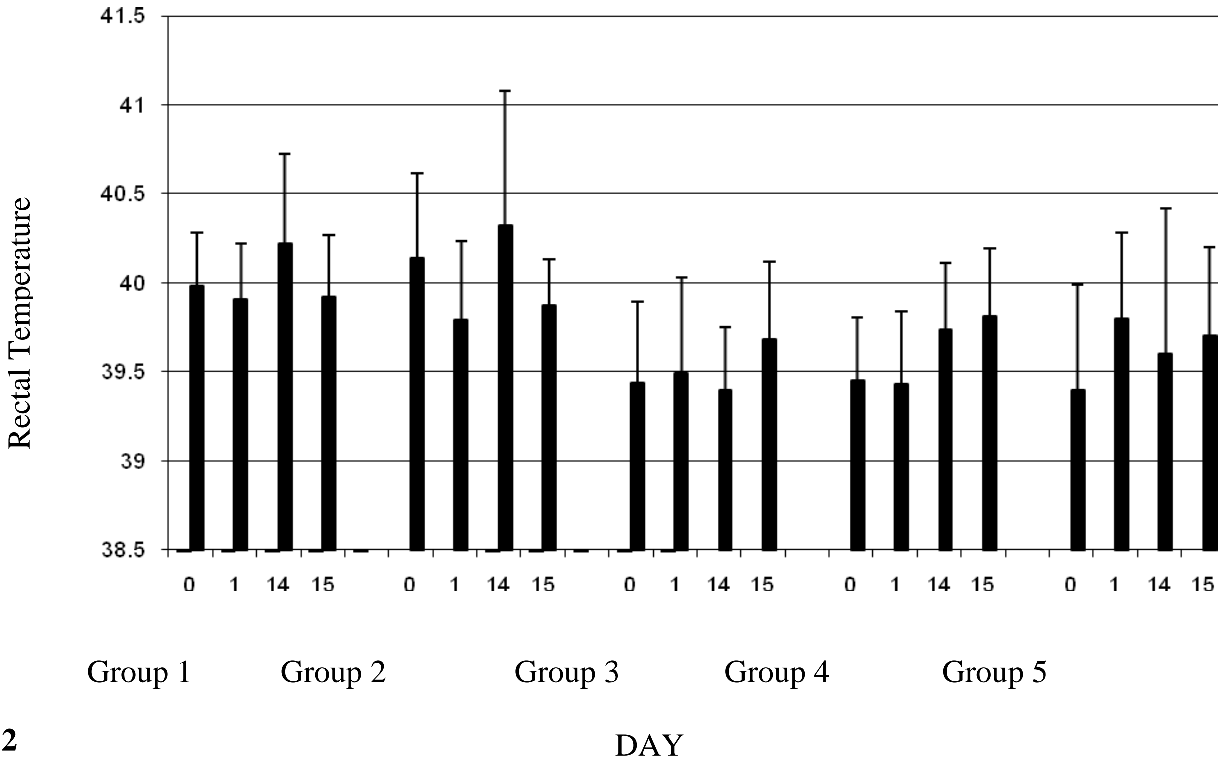

Fig. 2 ay 1), day of booster (Day 14) and 24 hours later (Day 15). Groups 1 and 2 had no maternal antibody at time of vaccination while groups 3, 4 and 5 did. Groups 2 and 4 were vaccinated with HA RP, groups 1 and 3 were vaccinated with placebo and group 5 was vaccinated with commercial vaccine. There were no significant temperature differences on day of vaccination compared with the following day.

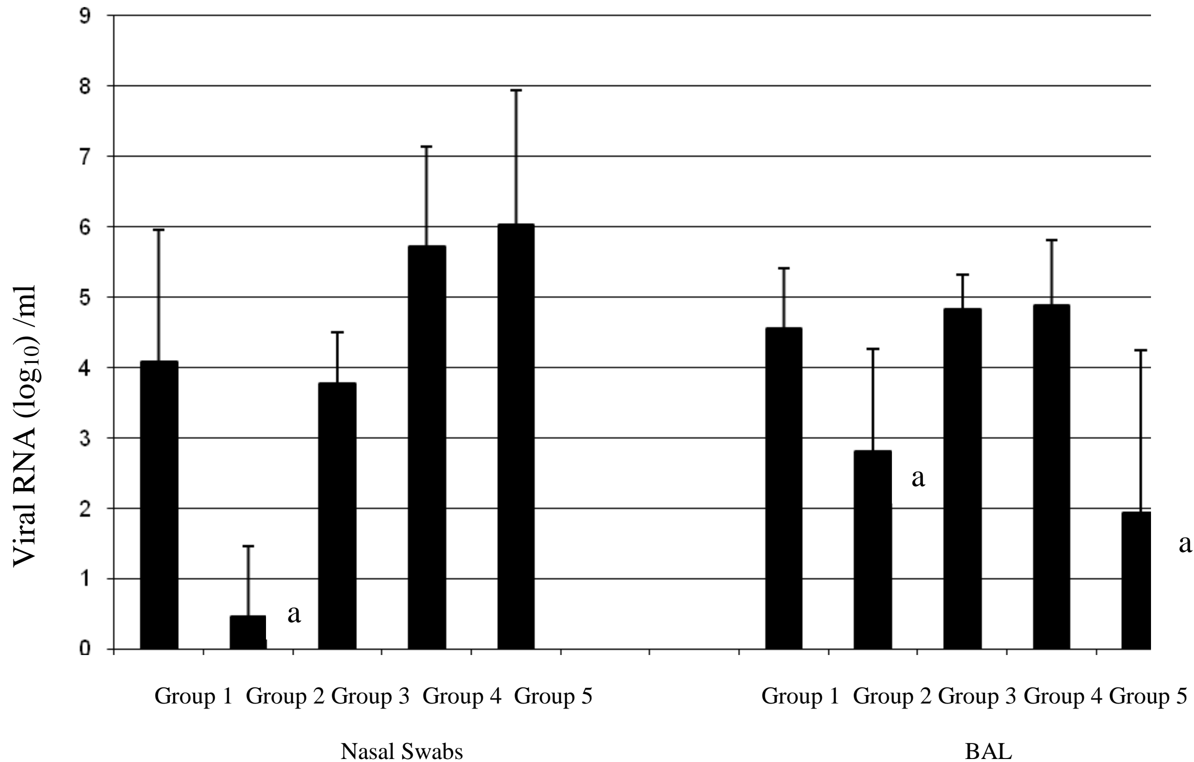

Fig. 3. Experiment 1. Mean RT-PCR log_{10} (+S.D.)/ml of influenza virus RNA 4 days (Day 57) after experimental challenge with H3N2. Groups 1 and 2 had no maternal antibody while groups 3, 4, and 5 did. Groups 2 and 4 were vaccinated with HA RP at day 0 and 14, groups 1 and 3 were vaccinated with placebo at day 0 and 14, and group 5 was given commercial vaccine at day 0 and 14. Significantly different viral RNA amounts ($P < 0.05$) in nasal swabs and BAL are designated with different letter superscripts.

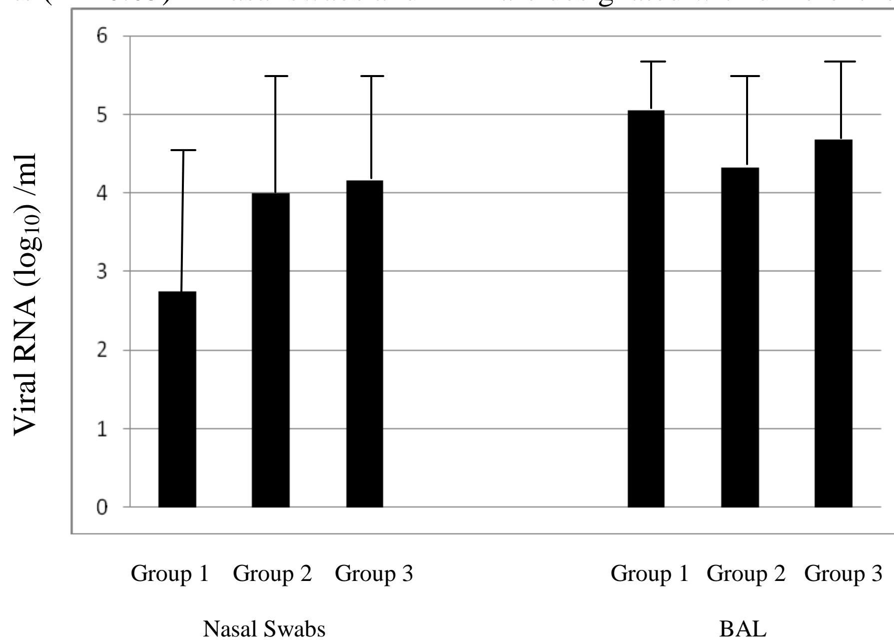

Fig. 4. Experiment 2. Mean RT-PCR log_{10} (+S.D.)/ml of influenza virus RNA 4 days (Day 57) after experimental challenge with H3N2. All groups had maternal antibody. Group 1 was vaccinated with placebo at day 0 and 14, group 2 was vaccinated with HA RP and NP at day 0 and 14, group 3 was vaccinated with HA RP and NP at day 0 and commercial vaccine at day 14. There were no significant differences between the groups.