Immunization for Influenza A Virus by Intranasal Administration of Alphavirus Replicon Particles

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Abstract

Improved vaccines are necessary to prevent swine influenza, especially in young growing pigs. The objective of this study is to determine whether intranasal vaccination with Alphavirus replicon particle (RP) vector vaccine prevents influenza A virus (IAV) in pigs. RP vaccine was prepared with the hemagglutinin (HA) gene of pandemic H1N1 influenza virus (A/California/04/2009, pH1N1). The efficacy of intranasal (IN) administration with pH1N1 HA RP was evaluated in two pig experiments. In the first experiment, prime/boost RP vaccination was administered IN/IN to pigs. In the second experiment, pigs were administered a one dose intramuscular (IM) or IN HA RP vaccine, or with a combination of IN/IM routes with an interval of three weeks. Results showed that two doses IN administration of HA RP did not protect pigs against IAV; one dose IM and combination IN/IM routes vaccination with HA RP reduced pneumonia significantly and partially inhibited virus shedding following homologous challenge.

Introduction

A major problem of vaccinating pigs with IAV vaccine is that maternal antibodies interfere with the vaccine efficacy. Mucosal vaccination has the potential to avoid maternal antibodies interfering with vaccine efficacy in piglets. Previous studies indicated that IN administrated vaccine could induce immune response and protect pigs. The objective of this study is to determine if intranasal vaccination with RP vector vaccine prevents IAV in pigs.

Methods and Materials

RP vaccine: RP vaccine was prepared with the HA gene of pH1N1.
Experiment 1: Fifteen IAV maternal antibody free pigs were vaccinated with RP. Prime/boost RP vaccination was administered IN/IN to pigs, with IM/IM administration as positive control and sham vaccination as negative control (five pigs each group) (Table 1). Three weeks post boost vaccination, all pigs were challenged by homologous virus.
Experiment 2: Twenty-five IAV antibody free pigs were involved. Five pigs in each group were administered with one dose HA RP vaccine IM or IN, or with a combination of IN/IM routes with an interval of three weeks (Table 2). Positive control and negative control groups received the same vaccination treatment as experiment 1. All pigs were challenged by homologous challenge following two doses vaccination.
Assays and observation: All pigs were killed and necropsied on the fifth day post challenge. Anti-HA antibody titer in serum was tested by Hemagglutination-inhibition (HI) test. Live virus was isolated from nasal swab and bronchoalveolar lavage (BAL) samples. Clinical signs including body temperatures and coughing were observed after challenge. Pathology examinations including gross lung lesion scores, histopathologic lung lesion scores and immunohistochemistry (IHC) staining were performed.

Results and Discussion

In the first experiment, pigs were not protected by IN/IN administration of RP against IAV challenge. Virus titers and pneumonia were not significantly reduced in the RP IN/IN administered group compared to the control group (Figure 1A). In the second experiment, in IN/IM or one dose IM administered pigs, from no more than two pigs at 2 DPC and 3 DPC, and no pigs at 3 DPC to 5DPC could virus be obtained. Live virus was detected in all five pigs from one dose RP IN group and the sham vaccinated group. We also found that one dose IM and combination of IN/IM vaccination with HA RP significantly reduced pneumonia lesions compared with sham vaccinated pigs (Figure 1B). Future study will evaluate whether IN/IM administration of RP will protect pigs against virus challenge, with the existence of IAV maternal antibodies in pigs.

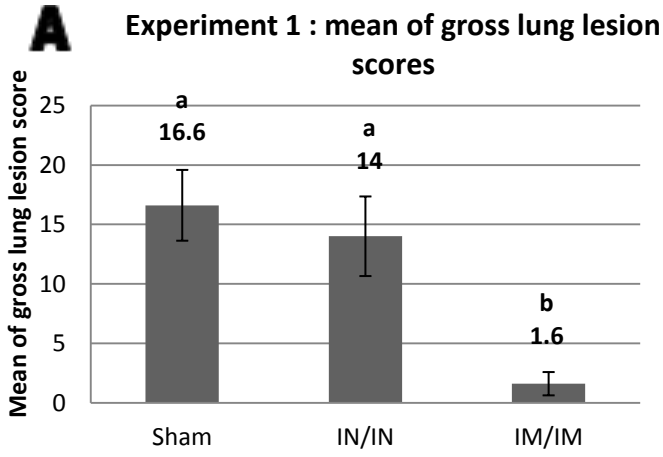
Table 1. Experiment 1 animal study protocol.

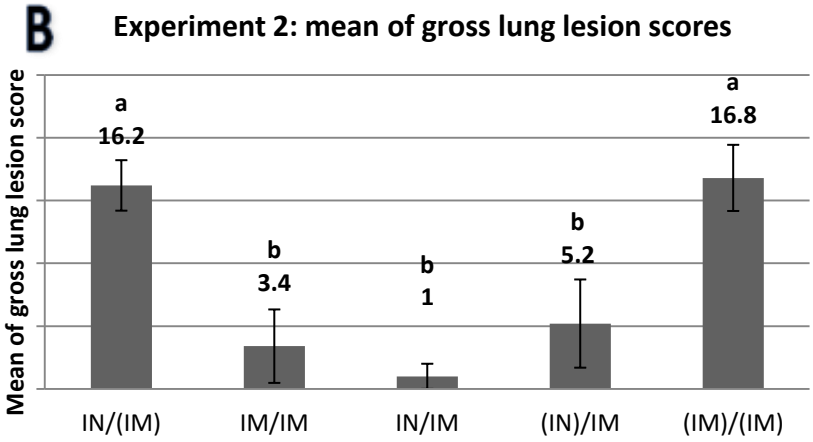
Group name	Pig numbers	Day 0 prime vaccination	Day 21 boost vaccination	Day 42 challenge	Day 47 necropsy pigs
Sham	5	IM Placebo	IM placebo	pH1N1 IAV	5
IN/IN	5	IN RP	IN RP	pH1N1 IAV	5
IM/IM	5	IM RP	IM RP	pH1N1 IAV	5

Table 2. Experiment 2 animal study protocol. Brackets indicate the use of placebo instead of RP vaccine in the specific route.

Group name	Pig numbers	Day 0 prime vaccination	Day 21 boost vaccination	Day 42 challenge	Day 47 necropsy pigs
IN/(IM)	5	IN RP	IM placebo	pH1N1 IAV	5
IM/IM	5	IM RP	IM RP	pH1N1 IAV	5
IN/IM	5	IN RP	IM RP	pH1N1 IAV	5
(IN)/IM	5	IN placebo	IM RP	pH1N1 IAV	5
(IM)/(IM)	5	IM placebo	IM placebo	pH1N1 IAV	5

Figure 1. Mean of gross lung lesion scores of each group. Volumes represent mean of lung scores with standard errors and same lowercase character mean no statistically significant difference. (A) Experiment 1, there was no significant difference between sham and IN/IN group, but IM/IM group was significantly lower than sham and IN/IN group. (B) Experiment 2, significant difference was observed between IN/(IM), (IM)/IM groups and other three groups.




B
Experiment 2: mean of gross lung lesion scores
Mean of gross lung lesion score
a
16.2
b
3.4
b
1
b
5.2
a
16.8
IN/(IM)
IM/IM
IN/IM
(IN)/IM
(IM)/(IM)