Evaluation of a recombinant human adenovirus-5 vaccine administered via needle-free device and intramuscular injection for vaccination of pigs against swine influenza virus

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Objective—To evaluate the safety and efficacy of a human adenovirus-5 vaccine for protecting weaned pigs against swine influenza virus subtype H3N2 infection when administered via 2 injection methods.

Animals—76 pigs.

Procedure—6 groups of weaned pigs received a 10-fold serial dilution of recombinant adenovirus expressing H3 hemagglutinin and a constant amount of recombinant adenovirus expressing nucleoprotein, either via a needle-free injection device or by traditional IM injection. In each group of 10 pigs, 1 served as a nonvaccinated contact pig to monitor whether there was spread of vaccinial virus from pig to pig. Vaccinated pigs and nonvaccinated controls were challenged or sham-inoculated 5 weeks later. After challenge, pigs were observed for clinical signs and nasal secretions were tested for virus. On day 5 after challenge, pigs were euthanatized; lungs were examined for gross lesions, and bronchoalveolar lavage specimens were tested for virus replication.

Results—A hemagglutination inhibition (HI) antibody response was elicited in a dose-dependent manner. Traditional IM administered vaccination induced consistently higher HI antibody responses than vaccination via needle-free injection, but the differences were not significant. Likewise, traditional IM administration was superior at reducing nasal virus shedding except at the highest dose, at which both methods blocked virus replication. The severity of lung lesions was reduced in a dose-dependent manner by both vaccination methods. Sentinel pigs did not seroconvert.

Conclusions and Clinical Relevance—The human adenovirus-5 vaccine at high doses prevented nasal virus shedding after challenge exposure with both methods of administration. The replication-defective vaccine virus was not transmitted to sentinel pigs. (*Am J Vet Res* 2005;66:1943–1947)

Swine influenza is an acute contagious viral disease of the porcine respiratory tract. The discovery of new subtypes and reassortant viruses in the United States has led to increased attention to the disease's impact on the swine industry and public health.[1-3] Clinical signs are usually followed by rapid recovery in individual animals and may include high fever, lethargy, anorexia, coughing, sneezing, nasal discharge, conjunctivitis, abortions, and, in some instances, low fatality rates in sows.[4-6] Classic **swine influenza virus (SIV)** subtype cH1N1 was the sole subtype responsible for disease in pigs in the United States until recently. In 1998, SIV subtype H3N2 emerged, and the subtypes and reassortants presently affecting pigs in the United States are reassortants rH1N1, H3N2, and H1N2.[7-9]

In response to the newly emerging SIV variants, new multivalent vaccines were manufactured and licensed commercially. All commercially available swine vaccines for SIV are killed vaccines that require a 2-dose administration regimen.

Broken vaccination needles that imbed in swine carcasses and inflammation or abscess formation at the site of injection are concerns for producers in the swine industry. A number of companies are testing and marketing needle-free injection devices for safe vaccination or pharmaceutical delivery.[10-12] The purpose of the study reported here was to test the efficacy of a human adenovirus-5–vectored SIV vaccine administered with a needle-free device in pigs and to compare that method with the efficacy of traditional needle and syringe (IM) administration. We also wanted to determine whether the replication-defective vaccinial strain of virus would spread to nonvaccinated contact cohorts.

Materials and Methods

Experimental design—The experiment included 76 pigs from a specific pathogen-free herd that were seronegative for antibodies against SIV. The pigs were weaned at 2 weeks of age, delivered to the National Animal Disease Center, and allowed to acclimate to their new environment and diet for 1 week. The experiment was carried out under the guidelines of the Animal Care and Use Committee.

Upon arrival, the pigs were randomly allocated to 8 groups. Six of the groups (10 pigs/group) were to be vaccinated, 1 group of 9 pigs was left unvaccinated for later challenge, and 1 group of 7 unvaccinated pigs was sham-inoculated at the time of challenge with porcine **bronchoalveolar lavage (BAL)** fluids free of SIV. Each group of pigs was housed separately in an individual animal isolation room. At 3 weeks of age, 6 groups of pigs were vaccinated with different doses, either IM with a needle and syringe or by use of a needle-free pneumatic injection device,[a] and 5 weeks later, the vaccinated pigs and a group of nonvacci-

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nated control pigs (group 2) were challenge exposed to SIV subtype H3N2.

For vaccinations, the recombinant adenovirus (Ad-HA-14.2) expressing the SIV H3 hemagglutinin[13] was administered in a 10-fold dilution series. Pigs in groups 7 and 8 received 2×10^{10} $TCID_{50}$/pig (high dose), those in groups 5 and 6 received 2×10^{9} $TCID_{50}$/pig (middle dose), and those in groups 3 and 4 received 2×10^{8} $TCID_{50}$/pig (low dose). Each 0.5-mL volume of vaccine suspension also contained a constant amount (2×10^{10} $TCID_{50}$/pig) of a second recombinant adenovirus (Ad-NP-13.4) that expressed the SIV nucleoprotein.[14]

Vaccinations were performed in the isolation rooms. Each tube of a given vaccine dilution was first administered (0.5 mL/pig) to pigs in the needle-free group. Vaccine doses were administered on the hairless medial side of the pig's right thigh via needle-free injection with a No. 3 syringe. Results of previous work had revealed that the No. 3 syringe was adequate for SC and IM delivery of solutions to pigs of this size. When all vaccinations in that room were completed, the same tube of diluted vaccine was used in the next room for administration via traditional IM injection (0.5 mL/pig) by use of a syringe and 25-gauge needle in the hamstring area of either hind limb. Pigs in groups 3, 5, and 7 were vaccinated via the needle-free technique, and pigs in groups 4,6, and 8 were vaccinated by use of a needle and syringe.

In each of the rooms housing vaccinated pigs, only 9 of the 10 pigs were vaccinated; 1 randomly selected pig was left unvaccinated to serve as a sentinel. Sentinels remained in close contact with vaccinated pigs for 5 weeks after administration of the vaccines.

Five weeks after vaccination, all vaccinated pigs and control pigs were challenge exposed via intratracheal instillation of 2.4 mL of porcine BAL fluids containing 4×10^{5} $TCID_{50}$ of SIV subtype H3N2/mL. Challenge virus was prepared from tissues from a herd of swine with severe respiratory tract disease that was submitted to the Iowa State University Veterinary Diagnostic Laboratory.[b] The challenge virus (H3N2) was prepared in pigs by serial passage of the virus in porcine respiratory tract tissues (lung homogenates and lung lavage fluids). The challenge virus and the SIV strain in the recombinant vaccine[13] were derived from different farms in Iowa but were closely related. The N-terminal HA1 portion of the challenge virus H3 gene was 99% identical at the level of deduced amino acids to the HA1 portion of the H3 gene expressed by the recombinant vaccine.[14]

For challenge exposure, pigs were anesthetized with a solution (1 mL/5.4 kg) containing 33 mg of tiletamine-zolazepam,[c] 44 mg of ketamine, and 22 mg of xylazine/mL of final solution[15]. A laryngoscope was used to pass a small-diameter tube over the epiglottis and through the larynx of the anesthetized pig, and an aliquot of fluid containing SIV was deposited in the trachea above the major bifurcation. To reduce the possibility of secondary bacterial infections, oxytetracycline[d] (20 mg/kg) was given IM at the time of challenge exposure and 2 days postchallenge. The 7 nonvaccinated pigs were sham-inoculated controls. At the time of challenge exposure, those pigs were inoculated via the same technique with BAL fluids that contained no SIV. All pigs were euthanized and underwent necropsy 5 days after challenge infection or sham inoculation.

Clinical signs that developed after challenge exposure were monitored by observing the pigs twice daily, and daily rectal temperatures were determined before challenge and for 4 days after challenge. To determine virus shedding, nasal swab specimens from each pig were collected daily on days 0 through 5 by use of moistened polyester-tipped applicators.[e] After swabbing, the applicators were submerged in 1 mL of transport medium consisting of McCoy 5A medium[f] supplemented with penicillin (25 U/mL), streptomycin (25 µg/mL), neomycin (25 µg/mL), bacitracin (0.25 U/mL), and gentamicin (50 µg/mL) and promptly frozen and stored at –80°C. On day 5 after challenge, control pigs and principals were euthanized, the lungs were retrieved and examined for gross lesions, and the degree of consolidation on the surface of each lung lobe was estimated visually. The transport medium was also used to collect 20 to 30 mL of BAL fluid from each pig.

Serum samples were collected on the day of vaccination and at 2, 4, and 5 weeks and 5 weeks plus 5 days after vaccination. The 5-week serum samples were collected before challenge exposure, and the 5-week plus 5-day serum samples were collected at the time of necropsy examination.

Hemagglutination inhibition assay—Hemagglutination inhibition (HI) tests were carried out in microtiter plates.[16,17] The virus used for hemagglutination in this test was identical to the source of the H3 and nucleoprotein genes used to construct the adenovirus vaccines. Serum samples were pretreated with a receptor-destroying enzyme.[g] For pretreatment, 50 µL of serum was mixed with 200 µL of the receptor-destroying enzyme (100 U/mL) and incubated overnight at 37°C. One hundred fifty microliters of a 2.5% sodium citrate solution was added, and the sample was heat-inactivated at 56°C for 30 minutes. Two hundred microliters of treated serum was mixed with 50 µL of a 50% solution of washed turkey RBCs and incubated for 30 minutes at 21°C. After this incubation, RBCs were separated via centrifugation ($800 \times g$ for 10 minutes at 4°C), and the supernatant, a 1:10 dilution of the original serum sample, was used in the HI test. Each serum sample was then serially diluted 2-fold in 0.01M PBS solution (pH, 7.4) and incubated with 4 hemagglutinating U/well. Turkey RBCs (0.5%) were added, mixed, and allowed to sit undisturbed for 45 minutes at 4°C. The HI titer was calculated as the reciprocal of the highest serum dilution yielding complete inhibition.

Virus titration—A 10-fold dilution series of nasal swab and lung lavage fluid specimens was prepared in McCoy 5A medium containing trypsin and antimicrobials (ie, medium supplemented with L-1-tosylamide-2-phenylethyl chloromethyl ketone–treated trypsin[h] [0.5 µg/mL], penicillin [25 U/mL], streptomycin [25 µg/mL], neomycin [25 µg/mL], bacitracin [0.25 U/mL], gentamicin [50 µg/mL], and amphotericin B [2.5 µg/mL]). Confluent Madin-Darby canine kidney cells in 96-well plates were rinsed twice with the same trypsin-containing medium. After the second rinse, medium was removed from the wells and 50 µL of sample was added to each well. Four wells were inoculated with each undiluted and 10-fold–diluted sample. Plates were incubated for 2 hours at 37°C, and samples were aspirated. Each well received 200 mL of trypsin-containing medium and was incubated with 5% CO_2 at 37°C. The plates were observed daily for cytopathic effects and were fixed with methanol and stained with crystal violet at the end of 6 days. Wells with positive results were scored, and the titer was determined by use of the Karber method for calculating 50% end point dilution.[18]

Statistical analyses—A 2-factor repeated-measures ANOVA was used to compare mean HI and viral titers in nasal swab specimens at each time-unit interval. If a significant ($P \leq 0.05$) difference among treatment groups was determined from the ANOVA, comparisons of treatment groups were performed with the Duncan multiple range test at the $\alpha = 0.05$ level for significant ANOVA F values. Statistical software[i] was used for analyses.

Results

Serologic response to vaccination and challenge—By 2 weeks after vaccination, there was a dose-depen-

dent HI response in all vaccinated pigs (**Figure 1**). In pigs in the low- and middle-dose groups, HI titers reached a plateau at 4 weeks after vaccination and were maintained at similar values 5 weeks after vaccination. With the higher dosage, however, HI titers continued to rise through the fifth week after vaccination.

At 5 weeks after vaccination, the geometric mean serum HI titers stimulated by traditional IM vaccination were increased at each dose, compared with mean values resulting from needle-free administration (**Table 1**), but the differences between injection methods within dosages were not significant.

Serum samples were collected only at 5 days after challenge, so it was not possible to determine if an anamnestic response occurred after vaccination. For naïve group 2 pigs (challenged controls), an HI titer of 20 developed 5 days after challenge (Figure 1). Overall, the 2 groups that received the low vaccine dose had the greatest percentage increases in serum HI titer by day 5 after challenge; the 2 middle-dose groups had intermediate increases in HI titer; and the smallest increase, or a mean value decrease for group 7, occurred at the highest vaccination dose.

Safety—The recombinant adenovirus vaccine viruses are replication-defective, so HI or nucleoprotein antigens should be expressed only in the initially infected index cells. To monitor this safety feature, 1 pig chosen at random was left unvaccinated in each of the 6 rooms, whereas the other 9 pigs in the group were vaccinated. The 6 sentinel pigs remained in contact with vaccinated pigs for 24 hours daily during the 5-week period after vaccination. All 6 sentinel pigs remained seronegative for antibodies against SIV for the 5-week interval, indicating a lack of spread of the vaccine viruses (Figure 1).

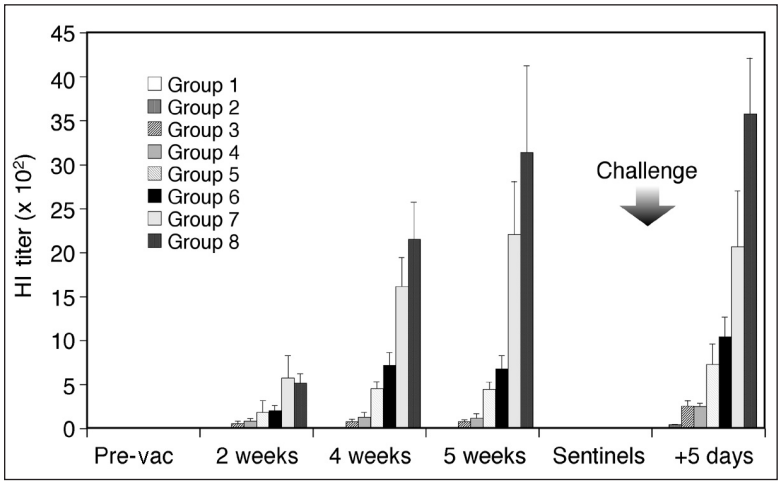

Figure 1—Geometric mean ± SE serum hemagglutination inhibition (HI) antibody titers in control pigs and pigs vaccinated with a human recombinant adenovirus-5 vaccine after vaccination and at 5 days after challenge with swine influenza virus. Group 1 = Sham-challenged controls. Group 2 = Challenged controls. Groups 3 and 4 = Low-dose vaccination via needle-free injection or hypodermic needle, respectively. Groups 5 and 6 = Middle-dose vaccination via needle-free injection or hypodermic needle, respectively. Groups 7 and 8 = High-dose vaccination via needle-free injection or hypodermic needle, respectively. Pre-vac = Prevaccination. Sentinels = Nonvaccinated nonchallenged pigs.

Table 1—Geometric mean ± SEM serum hemagglutination inhibition (HI) antibody titers measured at 5 weeks after vaccination in pigs (n = 9/group) vaccinated with a recombinant human adenovirus-5 vaccine via standard (IM) needle injection or a needle-free device.

Dose ($TCID_{50}$/pig)	Standard needle injection	Needle-free injection
Low (2×10^8)	107 ± 32	71 ± 18
Middle (2×10^9)	658 ± 132	447 ± 82
High (2×10^{10})	3,129 ± 980	2,204 ± 604

Clinical signs after challenge exposure—Intratracheal introduction of SIV-containing fluids was performed to induce acute disease. Except for 1 day on which there were high fevers in the control pigs, observed clinical signs were mild. On day 1 after challenge, intermittent coughing was observed in a few pigs. In general, most pigs, including control pigs, were alert and active and continued to eat. However, all control pigs were febrile (mean rectal temperature, 40.8°C) on day 1 after challenge, whereas all vaccinated pigs were afebrile on day 1 after challenge. Group 5 vaccinated pigs (middle dose, needle-free administration) had a mild increase in rectal temperature (mean, 40.1°C) on day 2 after challenge, with rectal temperatures ≥ 40°C for 6 of 9 pigs.

Virus shedding after challenge—Nasal shedding of virus was significantly reduced in vaccinated pigs, compared with nonvaccinated control pigs (**Figure 2**). The reduction in nasal shedding was dose-dependent, with the highest vaccine dose, administered either parenterally or via pneumatic injection, completely blocking nasal shedding of challenge virus. At the low and middle doses, administration via needle and syringe yielded superior results, compared with needle-free administration. At the low vaccine dose, needle-free administration yielded a significant reduction in virus shedding on days 1 and 5 after challenge, but the reduction in virus shedding on days 2, 3, and 4 after challenge was not significantly different than nasal shedding of virus observed in control pigs. For the standard needle and syringe method of delivery at the low dose, significant reductions in virus shedding were achieved on days 1, 2, 4, and 5, but not on day 3, after challenge. Nasal shedding in pigs that received the middle and high doses by both methods of administration was significantly reduced, compared with shedding from control pigs, on all days after challenge.

Fluids obtained via postmortem lavage of the airways were collected at the time of necropsy on day 5 after challenge. Swine influenza virus was recovered from each control pig, with a mean group titer of 5.24×10^2 $TCID_{50}$/mL. Virus was not recovered from vaccinated pigs except for 2 pigs in the group that received the middle dose with needle and syringe administration; in those pigs, minimal numbers of virus were detected.

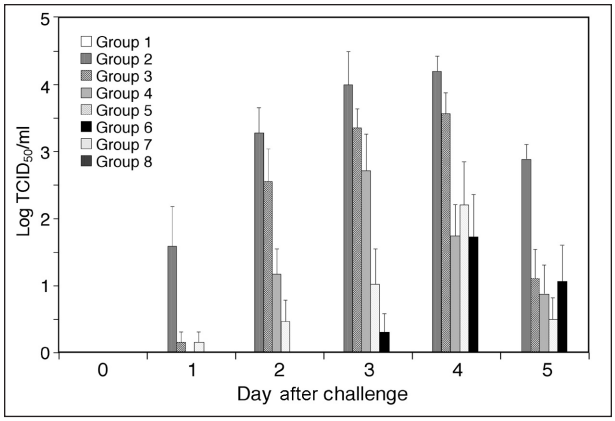

Figure 2—Geometric mean ± SE swine influenza virus titers measured from nasal swab specimens of control and vaccinated pigs after intratracheal challenge with 10^6 $TCID_{50}$ of swine influenza (H3N2) virus. *See* Figure 1 for remainder of key.

Table 2—Findings at necropsy in control pigs and pigs vaccinated with a recombinant human adenovirus-5 vaccine of various doses and via IM injection or a needle-free technique.

Vaccination group	Normal lungs	Mild* lesions	Moderate† lesions	Severe‡ lesions
Sham-inoculated controls	6	1	0	0
Challenged controls	0	0	1	8
Low dose, needle-free	2	3	3	1
Low dose, IM	3	2	4	0
Middle dose, needle-free	2	6	0	1§
Middle dose, IM	2	7	0	0
High dose, needle-free	4	5	0	0
High dose, IM	3	5	1	0

Data given as number of pigs in each lesion category.
*< 5% in any lung lobe. †5% to < 25% in any lung lobe. ‡≥ 25% in any lung lobe §This pig was considered unthrifty.

Gross lung lesions—The degree of lung involvement was low for all groups of vaccinated pigs, compared with the nonvaccinated controls, but some lesions, characterized by reddened areas of sharply demarcated consolidation, were not completely eliminated by vaccination (**Table 2**). For the highest and middle vaccine doses, most vaccinated pigs had mild lesions (ie, areas of consolidation that involved < 5% of any 1 lung lobe). At the lowest vaccine dose, most vaccinated pigs had mild or moderate lesions in the lungs. One pig in group 3 (lowest dose, needle-free administration) and another unthrifty pig from group 5 (middle dose, needle-free administration) had severe lung lesions. The latter pig was the runt in group 5 that also had a weak HI antibody response to vaccination.

Discussion

Vaccination of weaned pigs with a single dose of adenovirus-vectored vaccines was effective at protecting against infection with SIV subtype H3N2. Superior protective immunity derived from combining recombinant adenovirus vaccines, so that virus expressing the SIV hemagglutinin and virus expressing the SIV nucleoprotein are administered in each vaccine dose, has been reported.[14] In the trial of this report, efficacy at the high dose was evident in the elimination of clinical signs and prevention of virus shedding after challenge, but lung lesions still developed in pigs that received the high vaccine dose. Results from the present study complement and extend previous findings by indicating that the degree of immunity induced by vaccination is dose-dependent and, on the basis of results of sampling the sentinel pigs, that these replication-defective virus strains are safe and will not spread to nonvaccinated contact pigs.

The traditional IM method of vaccine administration with needle and syringe yielded consistently higher HI serologic responses than those induced after needle-free injection, but the differences in serum HI titers resulting from each method were not significant. Moreover, nasal virus shedding after challenge was completely blocked at the high dose by both vaccination methods, and at the middle dose, shedding was significantly reduced by both methods, with IM administration outperforming needle-free injection on all days except day 5 after challenge. At the low vaccine dose, virus shedding by vaccinates and controls was significantly reduced by the IM administration method on all days except day 3 following vaccination, whereas with the needle-free method, reductions in titer were significant only on days 1 and 5 after challenge. This finding was in contrast with other reports indicating that needle-free injection devices induced comparable levels of immunity in pigs.[10-12] To overcome swine carcass quality issues, further comparisons with various needle-free injection devices and routes of delivery are necessary.

Regarding efficacy of protection, pyrexia and replication of challenge virus in BAL fluids were prevented at all 3 doses of vaccine. The middle dose, however, more effectively reduced viral replication and shedding in the upper airways, reduced the severity of SIV-induced lung lesions, and elicited stronger HI antibody responses, compared with the low dose, although this was not analyzed statistically. Generation of HI antibodies in response to the low and middle doses was sufficiently low that there appeared to be a secondary HI antibody response to infection after challenge exposure. This was not the case at the high vaccination dose, a finding that may reflect the fact that immunity was at a sufficient level to block further replication of challenge virus, although mild lung lesions were observed in some pigs that were vaccinated with the high dose by both delivery methods.

The high vaccine dose was the most efficacious via delivery with either method; effective protection for immunologically naïve pigs was induced with a single dose on the basis of elimination of a febrile response and by prevention of virus replication and shedding in the upper and lower portions of the airways. The high dosage elicited a substantial HI antibody response that may have still been increasing when measured at 5 weeks after vaccination, whereas the responses after lower dosages peaked earlier. The severity of lung involvement was substantially less, but not completely eliminated, after administration of the high dosage, compared with the severity of challenged controls or pigs vaccinated at the low dosage. The severity of lung involvement was not substantially improved, compared with the severity of pigs vaccinated with the middle dosage.

A dose-dependent response was induced by administration of the adenovirus vector vaccine. Unlike the immune response to a modified-live virus vaccine, only 1 cycle of infection occurs with recombinant adenoviruses and antigen is synthesized and presented to the host's immune cells on the surface of the infected index cell. Thus, antigenic mass is limited with the replication-defective vectored vaccines so that the dose response is similar to that observed when killed vaccines are administered, rather than responses to vaccination with modified-live viruses that can continue to replicate in the vaccinated animal. However, all components of the immune response are potentially engaged because with vectored vaccines, immunogens are presented to host immune cells similarly to their presentation with modified-live virus vaccines.[19]

Nasal secretion of virus was blocked in pigs that received a single IM injection of the high vaccine dose, via either method of administration. Complete protection from nasal shedding after challenge is usually obtained only after natural infection with SIV and not by vaccination with commercial killed vaccines.[20] Vectored vaccines have the added advantage of inducing immunity in neonates and can also overcome interference by circulating maternally derived antibody.[21,22]

a. Biojector 2000, Bioject Medical Technologies Inc, Tualatin, Ore.
b. Kindly provided by Dr. Pat Halbur, Iowa State University, College of Veterinary Medicine, Ames, Iowa.
c. Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa.
d. Liquamycin LA-200, Pfizer Animal Health, Exton, Pa.
e. Daigger & Co Inc, Vernon Hills, Ill.
f. Gibco Invitrogen Corp, Carlsbad, Calif.
g. BioWhittaker Inc, Walkersville, Md.
h. Sigma Chemcial Co, St Louis, Mo.
i. SAS Institute Inc, Cary, NC.

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