Influenza virus of the H7N2 subtype has been introduced into noncommercial poultry in the United States, and this probably resulted in incidents of transmission of H7N2 virus to humans, documented in 2002 and 2003. This virus could be considered a potential threat to public health if it acquired person-to-person transmissibility. A favored approach for global pandemic preparedness is development of prepandemic vaccines for any potential pandemic virus. To this end, we created a high-growth reassortant virus (H7N2-PR8) containing the genes for the hemagglutinin and the neuraminidase from a low-pathogenicity (H7N2) virus strain and the remaining six genes from a human vaccine strain (H1N1). The reassortant strain was evaluated to assess its antigenicity, safety, and protective efficacy using a mouse model. Antigenicity studies using ferret antibodies raised against H7N2-PR8 indicated that this virus confers broad cross-reactivity with divergent H7 viruses of different years and lineages. Mice and chickens inoculated with high doses of H7N2-PR8 supported virus replication but survived, indicating that this virus is comparable to other avian viruses of low pathogenicity. To assess the protective efficacy of H7N2-PR8, mice were immunized with two doses of formalin-inactivated H7N2-PR8, alone or with alum. Vaccinated mice subsequently challenged with highly pathogenic viruses from homologous and heterologous lineages A/Canada/444/04 (H7N3) and A/Netherlands/219/03 (H7N7) showed pronounced reduction of wild-type virus replication. These studies indicate that H7N2-PR8 is immunogenic, safe, and protective in animal models; these are the essential attributes to qualify for phase I human clinical trials as a prepandemic vaccine.
MATERIALS AND METHODS
Parent virus strains, propagation, and quantification. The parent virus strain was isolated from an affected animal during the 2002 low-pathogenicity H7N2 avian influenza outbreak (1). A/Turkey/Virginia/4529/02 (TK/VA/02) was characterized by Immunology and Pathogenesis Branch, Influenza Division, CDC, Atlanta, GA. This virus was selected as the donor of H7 HA and N2 neuraminidase (NA) based on antigenic (hemagglutination inhibition [HI] and microneutralization tests) and genetic characteristics (low pathogenicity and absence of extra sequences at the HA cleavage site). A/Johannesburg/82/96-PR8-RESVIR-12 (Joh/PR8), provided by the Food and Drug Administration, was used as donor of the genes that encode the viral polymerases (PB2, PB1, PA), as well as NP, M, and NS (nucleotide sequences are available in the Influenza Sequence Database [19]). The five internal genes, encoding PB2, PB1, PA, M, and NS, are derived from A/PuertoRico/8/34 (PR8), whereas the NP, HA, and NA genes originate from A/Johannesburg/82/96 (Joh/96). The preparation of parent virus stocks and derivation of the reassortant seed were performed in accordance with Good Laboratory Practice guidelines. Virus stocks were propagated in the allantoic cavities of 10-day-old embryonated chicken eggs at 35°C for 48 h. The number of egg passages to generate and amplify reassortants was kept to a minimum to avoid spurious mutations. Virus stocks were prepared from seed previously subjected to two rounds of limiting dilution and stored at 80°C. Viral infectivity was determined by end point inoculation into embryonated eggs; the 50 percent egg infectious dose (EID50) was calculated by the method of Reed and Muench (25).

Generation of H7N2-PR8 high-yield reassortant virus. A volume of 1 ml of TK/VA/02 placed in a 35-mm cell culture dish was exposed to UV light (100 μJ/cm2) from a UV cross-linker (CL1000UVP; 254 nm; UVP) for 6 seconds. The virus was immediately mixed with Joh/PR8, and 0.1 ml was inoculated into 10-day-old embryonated eggs. The eggs were incubated at 35°C for 24 h; allantoic fluid was harvested, diluted 1:100, and incubated with ferret antisera against Joh/96 (H1N1). The mixture was incubated at room temperature for 40 min, and 0.1 ml was inoculated into embryonated eggs. After 24 h of incubation, the allantoic fluid was harvested and diluted 1:1,000 for a second incubation with ferret antisera. The mixture was inoculated into eggs, and after 48 h of incubation, allantoic fluid was harvested for RNA extraction using the QIAamp viral RNA minikit (QIAGEN, Valencia, CA). Genotyping was performed by reverse transcriptase PCR (RT-PCR) analysis performed using the One-Step RT-PCR kit (QIAGEN, Valencia, CA) and segment-specific oligonucleotide primers designed to detect exclusively the presence of either TK/VA/02 or Joh/PR8 genes (primer sequences are available upon request). Sequencing reactions were performed using a Big Dye Terminator, version 3.1, cycle sequencing kit (Applied Biosystems, Foster City, CA) and subtype-specific primers. Analyses of influenza virus gene sequences were performed using BioEdit, version 7.0.0 (10), and MEGA, version 3.1 (15).

HI tests. Ferret antisera against TK/VA/02 and H7N2-PR8 were produced for HI tests. For each virus, two adult lightly anesthetized female ferrets (Marshall Farms, North Rose, NY) were inoculated intranasally (i.n.) with 1 ml (104 EID50) of virus. Fourteen days after the first immunization, the ferrets were boosted on the footprint with 400 μl of a mixture containing 40 μg of sucrose gradient-purified virus with the synthetic adjuvant Titermax (CytRx Corporation, Norcross, GA). Blood was collected 2 weeks after boosting.

Ferret antisera against Joh/96 (H1N1) as well as the viruses A/Avan/11/23/05 (H7N2), A/Avian/NY/24/05 (H7N2), A/NewYork/107/03 (NY/107/03, H7N2), A/Canada/444/04 (CAN/04, H7N3), A/Chicken/Chile/180/54-02 (CK/Chile/02, H7N3), and A/Netherlands/219/03 (NL/219/03, H7N7) were provided by the Virus Surveillance and Diagnosis Branch, Influenza Division, CDC, Atlanta, GA. Prior to use in HI analyses, sera were treated with Vibrio cholerae NA (Denka- Seiken, Tokyo, Japan). HI tests were performed using turkey red blood cells, according to a WHO protocol (34).

Studies of pathogenicity in BALB/c mice. Mouse inoculations were performed as described by Chen et al. (6) and Lu et al. (18). Fifty percent mouse infective doses (MID50) of H7N2-PR8, TK/VA/02, and CAN/04 viruses were determined by inoculating groups of 6- to 8-week-old female BALB/c mice (Charles River Laboratories, Wilmington, MA). i.n. with 50 μl of serial 10-fold dilutions of virus. Four mice from each group were euthanized 4 days postinfection (p.i.) to harvest lung, brain, spleen, and nasal turbinate tissues to determine viral infectivity titers. The remaining mice (five per dilution) were examined daily for signs of disease (ruffling of feathers, respiratory distress) and weighed during a total period of 14 days. Log-transformed viral titers were compared using a two-tailed t test.

Protective efficacy studies with a mouse model. To prepare the inactivated H7N2-PR8 and TK/VA/02 virions for immunization, sucrose gradient-purified viruses were mixed with formalin (formaldehyde, 36.5 to 38%; Sigma) to a final concentration of 0.025% and kept at 4°C for 3 days. Loss of infectivity was established by two passages of the virus-formalin mixture in 10-day-old embryonated eggs.

The protective efficacy of vaccines was evaluated by intramuscular (i.m.) vaccination of groups of 6- to 8-week-old female BALB/c mice (eight mice/group) with 10 μg of vaccine alone, 10 μg of vaccine mixed with alum (2%; Alhydrogel; Superfos Biosseeds, Kristgaard, Denmark), phosphate-buffered saline (PBS) only, or PBS mixed with alum in a final volume of 0.1 ml. Mice received a second i.m. injection after an interval of 2 weeks. All groups of vaccinated mice were challenged by i.n. administration of 106 EID50 (100 MID50) of CAN/04 or NL/219/03 and monitored for signs of disease during 14 days. Three mice per group were euthanized at 4 days p.i. for tissue collection and virus titration. To determine antibody titers, bleeding from the retro-portal plexus was performed before the first and second immunizations as well as before termination of the study.

Studies of infectivity and pathogenicity in chickens. Pathogenicity tests were performed as described by Chen et al. (6). Briefly, groups of eight 4-week-old pathogen-free White Leghorn chickens were inoculated intranasally (33) with a standard dose (0.2 ml of a 1:10 dilution of stock virus) of H7N2-PR8 (107.9 EID50/ml) or TK/VA/02 (106.5 EID50/ml). For infectivity tests, the viruses were inoculated i.n. (0.1 ml, 106 EID50 [H7N2-PR8] and 105 EID50 [TK/VA/02]). The birds were observed daily for signs of illness. Oropharyngeal and cloacal swabs were collected 3 days after virus inoculation and on day 14 p.i. The birds were bled and euthanized 2 weeks after inoculation. The presence of influenza virus-specific antibodies was determined by the agar gel precipitin test.

Laboratory biosafety. All laboratory and animal studies that included infectious viruses of high pathogenicity or exotic avian viruses of low pathogenicity were conducted under approved biosafety level 3-enhanced protocols. Experiments using low-pathogenic North American viruses of avian origin (TK/VA/02 and H7N2-PR8) were conducted according to approved biosafety level 2 protocols.

RESULTS
Characterization of H7N2-PR8 reassortant virus. The viral progeny from coinfection of eggs followed by two egg passages in the presence of antibody to the H1N1 parental virus were evaluated by limiting dilution and RT-PCR with lineage-specific primers to determine their genetic makeup (genotype). Viruses containing two genes encoding the major antigenic proteins of TK/VA/02 (H7 HA and N2 NA) and the six genes from Joh/PR8 were identified. These reassortants replicated efficiently in eggs, and after two passages in embryonated eggs without antibody pressure (anti-H1N1), a vaccine seed stock was prepared by pooling different clones out of end point dilutions (1,024 hemagglutinating units/50 μl, 1010.25 EID50/ml).

The antigenic properties of the H7N2-PR8 reassortant virus were analyzed by HI test with a panel of antisera to homologous and heterologous viruses (Table 1). As expected, the titers obtained with homologous ferret antisera (against either

| Strain (subtype) | HI titer of ferret antisera against | % Amino acid identity of HA |
|-----------------|----------------------------------|-----------------------------|
| H7N2-PR8        | TK/VA/02 (H7N2)                  | 100                         |
| Avian/NY/23/05  | TK/VA/02 (H7N2)                  | 99.4                        |
| NY/107/03 (H7N2)* | TK/VA/02 (H7N2)                  | 98.4                        |
| Avian/NY/24/05  | TK/VA/02 (H7N2)                  | 97.2                        |
| CAN/04 (H7N3)*  | TK/VA/02 (H7N2)                  | 91.8                        |
| CK/Chile/02 (H7N3) | TK/VA/02 (H7N2)                  | 82.1                        |
| NL/219/03 (H7N7)* | TK/VA/02 (H7N2)                  | 79.9                        |

* a, virus with a history of infection in humans.

b Percent identity compared to TK/VA/02 HA.

* Percent identity compared to TK/VA/02 HA.

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FIG. 1. Daily body weight recordings after infection with escalating virus doses. In order to assess the pathogenicity of TK/VA (a), CAN/04 (b), and H7N2-PR8 (c), groups of mice were infected i.n. with six different dilutions of either virus and monitored daily for signs of mortality and morbidity during a period of 14 days. The data shown are from mice inoculated with the lowest, highest, and intermediate virus concentrations only and are expressed in log EID$_{50}$. 

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Can/04 (H7N7), TK/VA (H7N2), and NL/219/03 (H7N1) were used for challenge studies. The HI test used homologous HI antigens (β-propiolactone inactivated). Chickens were sacrificed 14 days after inoculation, and sera were harvested.

Pathogenicity and replication of reassortant virus in BALB/c mice. Mice infected with the parent virus TK/VA/02 (Fig. 1a) or H7N2-PR8 (Fig. 1c) did not show any signs of illness during the 14 days of observation. Mice inoculated with lower virus doses of TK/VA/02 or H7N2-PR8 (<7.2 and 7.9 log_{10} EID_{50}, respectively) gained weight during the experiment (Fig. 1). Modest and transient weight losses were observed on day 7 p.i. in mice inoculated with the highest doses of H7N2-PR8 (12% and 5% weight reductions) (Fig. 1c). However, these animals recovered weight within the next 24 h. All groups of mice infected with H7N2-PR8 showed higher virus titers in lung than mice infected with TK/VA/02 (2 log_{10} EID_{50} per ml difference; Table 2). There was no significant difference in viral titers between H7N2-PR8 and TK/VA/02 (<7.2 and 7.9 log_{10} EID_{50}, respectively) gained weight during the experiment (Fig. 1). Modest and transient weight losses were observed on day 7 p.i. in mice inoculated with the highest doses of H7N2-PR8 (12% and 5% weight reductions) (Fig. 1c). However, these animals recovered weight within the next 24 h. All groups of mice infected with H7N2-PR8 showed higher virus titers in lung than mice infected with TK/VA/02 (2 log_{10} EID_{50} per ml difference; Table 2). There was no significant difference in viral titers between H7N2-PR8 and TK/VA/02 (<7.2 and 7.9 log_{10} EID_{50}, respectively) gained weight during the experiment (Fig. 1). Modest and transient weight losses were observed on day 7 p.i. in mice inoculated with the highest doses of H7N2-PR8 (12% and 5% weight reductions) (Fig. 1c). However, these animals recovered weight within the next 24 h. All groups of mice infected with H7N2-PR8 showed higher virus titers in lung than mice infected with TK/VA/02 (2 log_{10} EID_{50} per ml difference; Table 2). There was no significant difference in viral titers between H7N2-PR8 and TK/VA/02 (<7.2 and 7.9 log_{10} EID_{50}, respectively) gained weight during the experiment (Fig. 1). Modest and transient weight losses were observed on day 7 p.i. in mice inoculated with the highest doses of H7N2-PR8 (12% and 5% weight reductions) (Fig. 1c). However, these animals recovered weight within the next 24 h. All groups of mice infected with H7N2-PR8 showed higher virus titers in lung than mice infected with TK/VA/02 (2 log_{10} EID_{50} per ml difference; Table 2). There was no significant difference in viral titers between H7N2-PR8 and TK/VA/02 (<7.2 and 7.9 log_{10} EID_{50}, respectively) gained weight during the experiment (Fig. 1). Modest and transient weight losses were observed on day 7 p.i. in mice inoculated with the highest doses of H7N2-PR8 (12% and 5% weight reductions) (Fig. 1c). However, these animals recovered weight within the next 24 h. All groups of mice infected with H7N2-PR8 showed higher virus titers in lung than mice infected with TK/VA/02 (2 log_{10} EID_{50} per ml difference; Table 2).
fected with TK/VA/02 via the either one of the two routes seroconverted.

**Immunogenicity and protective efficacy of formalin-inactivated H7N2-PR8 in mice.** In this study we compared the immune responses of mice to whole inactivated vaccines derived from TK/VA/02 or the H7N2-PR8 reassortant (Table 4). All mice immunized i.n. with inactivated vaccines developed detectable HI titers after a single dose; these titers were higher when the vaccine contained adjuvant. No HI antibody was detected in the sera of mice that received PBS or PBS plus alum. The second dose of vaccine resulted in substantial elevation of the titers elicited by the first dose, regardless of the presence or absence of adjuvant.

Each of the six groups of mice immunized with PBS, TK/VA/02, or H7N2-PR8, with or without alum as adjuvant, was divided at random into two and challenged with 100 MID$_{50}$ of the CAN/04 (H7N3) or NL/219/03 (H7N7) strain. CAN/04 replicated in the lungs of the PBS- and PBS-plus-alum-immunized groups (5 to 5.2 log$_{10}$ EID$_{50}$/ml), as well as in the nasal turbinates (1.4 to 2.6 log$_{10}$ EID$_{50}$/ml), without inducing any clinical signs of disease (Table 5). No mortality or weight loss was observed in any of the groups of immunized mice challenged with CAN/04, including the groups that received PBS or PBS plus alum (Fig. 2a).

Vaccination with H7N2-PR8 virus alone protected mice from infection in the lungs, but CAN/04 was isolated from nasal turbinates (2.5 log$_{10}$ EID$_{50}$/ml; Table 5) and spleen (1.2 log$_{10}$ EID$_{50}$/ml) in one out of three mice. Mice immunized with H7N2-PR8 plus alum showed complete protection in the lungs (Table 5). Low-titer CAN/04 virus was detected in the nasal turbinate of one mouse (1.3 log$_{10}$ EID$_{50}$/ml). No virus was detected in spleen or brain. In mice immunized with TK/VA/02 plus alum, no virus was detected in any of the tissues. However, in the group of mice immunized without alum, virus was isolated from lungs (3.7 log$_{10}$ EID$_{50}$/ml) and nasal turbinates (1.7 log$_{10}$ EID$_{50}$/ml).

Weight loss and mortality were observed at days 5 and 6 p.i. in

### Table 4. Immunogenicity of formalin-inactivated vaccines prepared against H7N2-PR8 and TK/VA

| Immunogen  | Use of alum | H7N2-PR8 | TK/VA | H7N2-PR8 | TK/VA |
|------------|-------------|----------|-------|----------|-------|
|            |             | CAN/04  |       | NL/219/03 |       |
|            |             | Dose 1  | p.c.  | Dose 1  | p.c.  |
|            |             | Dose 1  | p.c.  | Dose 1  | p.c.  |
| PBS        | No          | <20     | <20   | <20     | <20   |
|            | Yes         | 20      | 80–160| 20      | 80    |
| H7N2-PR8   | No          | 40      | 640–1,280| 40     | 1,280 |
|            | Yes         | 20      | 80–160| 20      | 80    |
| TK/VA      | No          | 20      | 320–1,280| 20     | 320–1,280 |
|            | Yes         | 20      | 20–1,280| 20     | 20–1,280 |

* Groups of mice received two doses of 10 µg of formalin-inactivated vaccine or PBS with or without alum i.m. 2 weeks apart.

### Table 5. Protective efficacy of formalin-inactivated H7N2-PR8 reassortant vaccine

| Immunogen  | Use of alum | Challenge virus | % Body wt on day 14 postchallenge | Challenge virus titers in: |
|------------|-------------|----------------|----------------------------------|---------------------------|
|            |             |                | Lung | Turbinate | Brain | Spleen |
| PBS        | No          | CAN/04         | 100  | 104      | 5.0 ± 0.46 | 1.4 ± 0.17 | <0.8 | <0.8 |
|            | Yes         | 100            | 105  | <0.8     | 5.2 ± 0.64 | 2.6 ± 0.12 | <0.8 | <0.8 |
| H7N2-PR8   | No          | 100            | 106  | <0.8     | 2.5 ± 0.64* | <0.8 | 1.2 (1/3) |
|            | Yes         | 100            | 104  | <0.8     | 1.3 (1/3)* | 1.7 (2/3)* | <0.8 | <0.8 |
| TK/VA      | No          | 100            | 103  | <0.8     | <0.8* | <0.8 | <0.8 |
|            | Yes         | 100            | 103  | <0.8     | <0.8* | <0.8 | <0.8 |
| PBS        | No          | NL/219/03      | 0    | 0        | 6.5 ± 0.4 | 1.6 (2/3) | <0.8 | 1.8 ± 0.42 |
|            | Yes         | 100            | 102  | <0.8     | 6.8 ± 0.17 | 4.7 ± 2.36 | 2.0 (1/3) | 1.4 (2/3) |
| H7N2-PR8   | No          | 100            | 100  | <0.8     | 2.5 (2/3)* | 4.4 (2/3)* | <0.8 | <0.8 |
|            | Yes         | 100            | 102  | <0.8     | <0.8* | <0.8 | <0.8 |
| TK/VA      | No          | 100            | 103  | <0.8     | <0.8* | <0.8 | <0.8 |
|            | Yes         | 100            | 102  | <0.8     | <0.8* | <0.8 | <0.8 |

* Groups of mice (n = 8) were injected i.m. with two doses of 10 µg of formalin-inactivated vaccine alone, vaccine with 1% alum, or PBS alone, 2 weeks apart.

* Mice were challenged i.n. 4 weeks after the second vaccine dose with 100 MID$_{50}$ of CAN/04 or NL/219/03.

* Five out of eight mice were monitored daily for 14 days after challenge. The rate of survival was determined at the end of the 14-day observation period.

* Three out of eight mice were sacrificed on day 4 postchallenge, and virus titers in different organs were determined. Each virus titer is expressed as the mean log$_{10}$ EID$_{50}$/ml ± standard deviation for organs with positive virus isolation. The lower limit of detection of virus in organs was 1.5 log$_{10}$ EID$_{50}$/ml. The numbers in parentheses indicate the number of mice in which <0.8 log$_{10}$ EID$_{50}$/ml virus was detected/total number of mice.

* Lung titers significantly different compared with PBS controls (P < 0.05).

* Lung titers not significantly different compared with PBS control (P > 0.05).
mice that were immunized with PBS or PBS plus alum and challenged with the highly pathogenic NL/219/03 strain (Fig. 2b). These groups had high virus titers in the lungs and nasal turbinates at 4 days p.i. (6.5 to 6.8 \log_{10} \text{EID}_{50}/ml and 1.6 to 4.7 \log_{10} \text{EID}_{50}/ml, respectively). Virus was detected also in brain tissues (2.0 \log_{10} \text{EID}_{50}/ml in one mouse) and spleens (1.8 to 1.4 \log_{10} \text{EID}_{50}/ml). In contrast, mice immunized with either H7N2-PR8 or TK/VA/02 in the presence or absence of alum did not reveal any signs of disease or weight loss after challenge with NL/219/03 (Fig. 2b). Both groups of mice that received adjuvanted vaccines were fully protected from challenge with NL/219/03 (Table 5). No virus was detected in lungs, nasal turbinates, spleens, or brains. Similar results were obtained in mice immunized with TK/VA/02 without adjuvant. However, in the group of mice immunized with H7N2-PR8 without adjuvant, virus was present in lungs (2.5 \log_{10} \text{EID}_{50}/ml) and nasal turbinates (4.4 \log_{10} \text{EID}_{50}/ml).

**DISCUSSION**

Several incidents of transmission of H7 subtype influenza viruses (H7N2, H7N3, and H7N7) from avian to humans have been reported in recent years (4, 9, 24, 32). Most infections involved poultry workers who were in close contact with infected birds during the outbreaks. Evidence of human-to-human transmission was also reported (14). Therefore, these viruses represent a risk for the human population and a potential for pandemic alert.

We selected TK/VA/02 as a donor of HA for reassortant vaccine because it is very similar to viruses that circulate in the farms that supply the New York live-bird markets (26). This HA lacks multiple basic amino acids at the cleavage site, eliminating the need for genetic manipulation before creating the reassortant. Nucleotide and amino acid analysis of HA sequences from H7N2 strains circulating in the United States showed that TK/VA/02 HA has at least 97% identity with the HA of currently circulating H7N2 North American virus strains. A high-growth H7N2-PR8 reassortant was produced in a Good Laboratory Practice laboratory using TK/VA/02 and Joh/PR8 to provide the two genes encoding HA and NA and the remaining six genes, respectively, through a conventional genetic-reassortment method (13). Antigenicity tests using ferret antiserum raised against H7N2-PR8 revealed that this serum exhibited the same reactivity as the antisera raised against TK/VA/02. Both antisera reacted to a panel of distinct H7 strains and lineages, which were isolated from different geographic regions.

Pathogenicity tests revealed that H7N2-PR8 and the parent

![Figure 2](http://cvl.asm.org/)
virus, TK/VA/02, are viruses of low pathogenicity in mice and chickens. Both viruses were able to replicate in lungs and nasal turbinates of mice. H7N2-PR8 replicated at higher titers in mouse lungs than TK/VA/02, probably because the genes were derived from PR8, a virus highly adapted to replication by multiple laboratory passages in mice. By contrast, i.n. inoculated TK/VA/02 parent virus replicated to higher titers in chickens than H7N2-PR8, probably the result of the PR8 backbone, which has been shown to completely inhibit or severely restrict influenza A virus replication in chickens (6, 30).

Immunization of mice i.m. with the formalin-inactivated whole virus vaccines against H7N2-PR8 or TK/VA/02 elicited the production high titers of HI antibodies, detected 4 days after the mice were challenged. The presence of alum increased the humoral immune response significantly for both viruses and correlated with the protection against challenge. The groups of mice immunized with H7N2-PR8 or TK/VA/02 containing alum were protected against the two heterologous strains isolated in humans, the North American H7N3 strain, CAN/04, and the Eurasian H7N7 strain NL/219/03.

The pathogenicity of CAN/04 was assessed in mice, revealing that, unlike NL/219/03, this virus did not cause signs of pathogenicity in these animals but replicated at high titers in the lungs. The clinical signs observed in humans infected with CAN/04 or related virus can probably explain the mild pathogenicity that was observed in mice. In humans, conjunctivitis and other milder flu-like symptoms developed during infection (12).

H7N2-PR8 virus has been shown to be of low pathogenicity in mammalian and avian animal models. Vaccination was protective in mice after lethal challenge using a heterologous virus. Ferret and mice antibodies generated against the whole-virus vaccines against H7N2-PR8 or TK/VA/02 elicited the increased humoral immune response significantly for both viruses and correlated with the protection against challenge.

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