NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
J Infect Dis. Author manuscript; available in PMC 2010 January 11.

Published in final edited form as:
J Infect Dis. 2009 March 1; 199(5): 711–716. doi:10.1086/596558.

A Live Attenuated H9N2 Influenza Vaccine Is Well Tolerated and Immunogenic in Healthy Adults

Ruth A. Karron[1], **Karen Callahan**[1], **Catherine Luke**[2], **Bhagvanji Thumar**[1], **Josephine McAuliffe**[2], **Elizabeth Schappell**[1], **Tomy Joseph**[2], **Kathleen Coelingh**[3], **Hong Jin**[3], **George Kemble**[3], **Brian R. Murphy**[2], and **Kanta Subbarao**[2]

[1]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore [2]Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda [3]MedImmune, Gaithersburg, Maryland

Abstract

Development of live attenuated influenza vaccines (LAIV) against avian strains with pandemic potential is an important public-health strategy. Either 1 or 2 10^7-$TCID_{50}$ doses of H9N2 LAIV A/chicken/Hong Kong/G9/97 were administered intranasally to 50 adults in isolation; 41 participants were H9N2 seronegative, 24 of whom received 2 doses. The vaccine was well tolerated; vaccine shedding was minimal. After 2 doses, 92% of H9-seronegative participants had ≥4-fold increases in hemagglutination-inhibition antibody, and 79% had ≥4-fold increases in neutralizing antibody; 100% had responses detected by at least 1 assay. Although replication of the H9N2 LAIV was restricted, 2 doses were immunogenic in H9N2-seronegative adults.

The emergence of H5N1, H7N7, and H9N2 avian influenza A subtypes in humans and the intercontinental spread of H5N1 influenza have made development of vaccines against these novel influenza viruses a global priority. Ideally, these vaccines would be antigen sparing and able to be produced rapidly, to induce cross-protective immunity to antigenically drifted strains, and to be delivered by individuals with minimal training.

Live attenuated influenza vaccines (LAIV) for pandemic influenza viruses could potentially meet many of these requirements. Several LAIVs containing avian hemagglutinin (HA) and neuraminidase (NA) genes and internal protein genes of cold-adapted A/Ann Arbor/6/60 H2N2 (AA *ca*) have been evaluated in preclinical studies [1-3]. In the present study, we describe the first clinical trial of a LAIV containing avian HA and NA and the 6 internal protein genes of AA *ca*, A/chicken/Hong Kong/G9/97 H9n2.

Participants, materials, and methods

This open-label study was conducted at the isolation unit and outpatient clinic of the Center for Immunization Research of the Johns Hopkins Bloomberg School of Public Health during 2005–2006. The clinical protocol and its revisions were approved by the Institutional Review

---

© 2009 by the Infectious Diseases Society of America. All rights reserved.

Reprints or correspondence: Dr. Ruth A. Karron, Center for Immunization Research, Hampton House 217, Johns Hopkins Bloomberg School of Public Health, 624 N. Broadway, Baltimore, MD 21205 (rkarron@jhsph.edu)..

Potential conflicts of interest: R.A.K. has been a consultant to and a member of an advisory board for Medlmmune. K.C., H.J., and G.K. are employees of Medlmmune.

Trial registration. ClinicalTrials.gov identifier: NCT00110279.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Board (IRB) of the Johns Hopkins Bloomberg School of Public Health. Informed, witnessed, written consent was obtained from each participant. Healthy men and nonpregnant women born after 1968 were enrolled because we wished to include only individuals who were unlikely to have cross-reactive antibodies to H9N2 influenza [4].

The 6:2 reassortant vaccine seed virus was generated as described elsewhere [1], by reassortment of wild-type (*wt*) H9N2 G9 A/chicken/Hong Kong/G9/97 with AA *ca*, at the Influenza Branch of the Centers for Disease Control and Prevention in Atlanta. The clinical trial material, Lot H9N2 G9/AA *ca*, manufactured by Novavax, had a mean infectivity of $10^{8.2}$ $TCID_{50}$/mL.

The study was conducted between 1 April and 20 December 20 of each year, when *wt* influenza was unlikely to be present. Participants were not enrolled if there had been at least 3 influenza hospitalizations at Johns Hopkins Hospital during the preceding week.

Several IRB-approved protocol modifications were made between 2005 and 2006. The original study called for a subset of individuals to receive 2 vaccine doses; however, in 2006 all individuals who consented received a second dose 4–6 weeks after the first dose. Also, participants enrolled during 2005 were not screened for hemagglutination-inhibition (HI) antibody to H9N2; however, because 9 participants had preexisting H9 HI antibodies, screening was initiated during 2006, and those with H9 HI antibody titers ≤1:8 were enrolled. Finally, the inpatient stay was shortened from 14 days in 2005 to 10 days in 2006, if discharge criteria were met (see below).

Medical histories, physical examinations, and laboratory tests were performed as described elsewhere [5]. Participants were admitted 2 days before vaccination, to allow them to become oriented to the isolation unit, and were monitored for acute illness. Those who were ill or uncomfortable with the isolation-unit procedures were discharged without being vaccinated.

On day 0, each participant received 0.5 mL of vaccine administered as nose drops. Clinical evaluations were performed [6] and nasal-wash (NW) specimens were obtained before vaccination and then daily until the participant was discharged. In the event of respiratory or febrile illnesses, NW specimens were cultured for other respiratory viruses [5].

Discharge of a participant was contingent on absence of vaccine virus, as determined by real-time reverse-transcriptase chain reaction (rRT-PCR), from NW specimens obtained for 3 consecutive days before discharge. No participant was required to stay in the isolation unit longer than anticipated.

Participants returned to the clinic on days 21, 28, and 42 after administration of each dose, for clinical assessment and to provide blood samples and NW specimens (days 28 and 42 only) for antibody testing.

NW specimens were tested for vaccine virus by quantitative culture [6] and by a modified rRT-PCR assay that amplified a portion of the influenza A M2 gene [7]. The Nuclisens Mini-MAG system (bioMerieux) was used for RNA extraction. The sensitivity of the rRT-PCR was ~10^{1} $TCID_{50}$/mL.

Sera were tested for H9N2 HI antibodies, by use of turkey red blood cells [6], and for neutralizing antibodies, by a modified microneutralization assay [8,9]; those with anti-H9 HI antibody titers >1:8 were considered to be H9 seropositive. IgG antibody to recombinant H9 G1 HA was measured by ELISA [6]. NW specimens were concentrated [6] and then were tested by use of the same antigen, to measure vaccine-specific IgA by ELISA [6].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Results

Of 134 participants who were screened, 50 were vaccinated; 23 received 1 dose of vaccine, and 27 received 2 doses of vaccine. Of the 50 participants who were vaccinated, 41 were H9 seronegative, and 24 of them received 2 doses of vaccine. Data from H9-seropositive participants are reported separately from those from H9-seronegative participants.

Of the 9 H9-seropositive participants, 3 received 2 doses of vaccine. After administration of dose 1, 3 participants (33%) reported headache and 1 reported myalgia; after administration of dose 2, 1 participant (11%) reported headache and myalgia (all cases of illness were grade 1; see table 1). Vaccine virus was not recovered by culture but was detected by rRT-PCR on day 1 in 2 participants (22%) after administration of dose 1 and on day 1 in 1 participant (33%) after administration of dose 2 (table 1). Of the 6 participants who received only 1 dose, 1 had ≥4-fold increases in neutralizing antibody and in NW IgA antibody to H9 HA (table 2). Of the 3 participants who received 2 doses, 1 (33%) had a ≥4-fold increase in serum IgG antibody titer after administration of dose 2 (table 2). When the response to dose 1 and the response to dose 2 are considered together, 1 participant (33%) had a ≥4-fold increase in serum HI antibody titer and 2 each (66%) had a ≥4-fold increase in neutralizing-antibody titer, in serum IgG antibody titer, and in nasal IgA antibody titer (table 2). In each of the 3 H9-seropositive participants (100%) who received 2 doses, an antibody response to the vaccine virus was detected by at least 1 method (table 2).

H9N2 G9/AA *ca* was well tolerated in the 41 H9-seronegative participants. After administration of dose 1, the following minor (severity grade 1) illnesses were observed: a single day of low-grade fever in 1 participant (2%), rhinorrhea in 4 (10%), conjunctivitis in 1 (2%), cough in 1 (2%), myalgias (systemic illness) in 3 (7%), and headache in 10 (24%) (table 1). After administration of dose 2 (to 24 H9N2-seronegative participants), 3 (13%) reported headache, and 1 (4%) had an episode of epistaxis.

Replication of H9N2 G9/AA *ca* was significantly restricted in H9N2-seronegative participants (table 1). After administration of dose 1, 2 participants shed virus that was detected, at titers of $10^{1.25}$ and $10^{0.75}$ $TCID_{50}$/mL of NW specimen, by culture on day 1. After administration of dose 2, vaccine virus was not detected by culture. Vaccine virus was detected by rRT-PCR in 15 participants after administration of dose 1 (in 14 participants on day 1 and in 1 participant on days 1 and 2) and in 2 participants after administration of dose 2 (in 1 participant on day 1 and in 1 participant on day 2).

Although its replication was significantly restricted, H9N2 G9/AA *ca* induced antibody responses in all participants who received 2 doses of vaccine virus (table 2). After administration of dose 1, HI antibody responses were detected in 29% of H9-seronegative participants, neutralizing antibody responses were detected in 24%, serum IgG responses were detected in 12%, and a NW IgA response was detected in 2%; antibody responses were observed in a greater proportion of participants after administration of dose 2 (table 2). When, in the 24 H9N2-seronegative participants who received 2 doses of vaccine virus, the response to dose 1 and the response to dose 2 are considered together, ≥4-fold increases in serum antibody titers were detected in 92% by HI assay, in 79% by microneutralization assay, and in 50% by IgG ELISA; 100% had responses detected by serum HI and/or neutralizing-antibody assays (table 2), 42% developed HI antibody titers of 4.0 $\log_2$ (1:16), and 38% had titers ≥5.0 $\log_2$ (≥1:32).

Discussion

In the present study, which is the first clinical evaluation of LAIV containing avian HA and NA, the A/chicken/Hong Kong/G9/97 (H9N2) LAIV was well tolerated and immunogenic,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

inducing ≥4-fold increases in either HI or neutralizing antibody in 100% of H9-seronegative individuals after they had received 2 doses of vaccine.

The present study of a H9N2 LAIV follows 3 previous studies of vaccines of inactivated H9N2 influenza. In the first of those studies, serum HI antibody titers ≥1:40 were achieved in ~70% of 18–60-year-old subjects who received 2 doses of whole-virus A/HK/1073/99 H9N2 vaccine administered with or without alum [10]. In the second study, 2 doses of whole-virus or subunit-A/HK/1073/99 H9N2 vaccine containing 7.5–30 μg of HA were administered, without adjuvant, to 18–60-year-old subjects. When the analysis was stratified by age, it was apparent that individuals born after 1968 would require 2 doses of either vaccine and that even this treatment would not fulfill all Committee for Proprietary Medicinal Products criteria for licensure [4]. A third study evaluated a subunit H9N2 G9 influenza vaccine administered, with or without MF-59, at doses of 3.75–30 μg of HA [9]. That study, which enrolled participants born after 1970, found that addition of MF-59 yielded HI antibody seroconversion rates of 75%–92% after administration of 1 dose of vaccine and 92%–100% after administration of 2 doses. Thus, the seroconversion rate after injection of 2 doses of the MF-59-adjuvanted vaccine was comparable to that observed after administration of 2 intranasal doses of H9N2 G9/AA *ca*. Although the HI antibody titers were higher in recipients of the MF-59-adjuvanted vaccine, this result does not necessarily indicate that the inactivated vaccine would afford better protection than does LAIV, because (1) the latter typically induces lower titers of serum antibodies than does the former [11] and (2) LAIV-induced protection is presumed to be mediated by induction of a variety of immunological mechanisms, including local antibody and cellular immunity [11,12]. Additionally, ~30% of participants in the study of MF-59 vaccine were H9 seropositive. Because the analysis was not stratified by H9 serostatus, immunologic priming may have contributed to the antibody titers achieved [4].

There were several unexpected findings in the present study. Although we restricted enrollment to individuals born after 1968, we found that 30% were H9 seropositive, a rate comparable to that seen in a study of H9N2 G9-lineage inactivated-virus vaccine administered to subjects born after 1970 [9]. These data suggest that recipients of experimental H9N2 influenza vaccines should be screened for the presence of preexisting H9 HI antibody.

We also did not anticipate the dissociation between detection of the vaccine in NW specimens and induction of antibody response. When monovalent influenza A/AA *ca* viruses are administered, vaccine virus can be recovered, by culture, from 40%–80% of individuals who are naive to the given influenza subtype, and the mean peak titers are $10^{1.5}$–$10^{3.0}$ $TCID_{50}$/mL of NW specimen [13]. In the present study, low titers of vaccine were detected by culture in 5% of H9 influenza-naive individuals after administration of 1 dose (table 1). Even when NW specimens were tested by rRT-PCR, vaccine virus was detected in 37% of H9-seronegative individuals after administration of dose 1 and in 8% after administration of dose 2 (table 1). Because all but 2 of these rRT-PCR-positive specimens were obtained on day 1 of the study, we cannot exclude the possibility that we were detecting input virus.

Included among several possible explanations for the significantly restricted replication of the H9N2 G9/AA *ca* vaccine virus are the following: (1) preexisting antibody to the human N2 NA cross-reacted with the avian N2 NA and decreased replication of vaccine virus; (2) preexisting cytotoxic T cell responses to internal viral proteins hastened viral clearance [14]; and, most likely, (3) the constellation of the avian influenza HA and NA genes combined with internal protein genes from a human influenza virus resulted in a vaccine with a host-range-restricted pheno-type for humans.

Our inability to detect replicating vaccine virus raises the possibility that the immune responses that we observed are related to the intranasal deposition of HA protein. However, 2 $10^{7.0}$

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

$TCID_{50}$ doses of H9N2 G9/AA *ca* vaccine are calculated to contain ≤0.4 μg of HA, on the basis of the HA-trimer-formula weight, the number of trimers per virion, and an estimate of the noninfectious/infectious ratio, the latter of which was derived from a comparison of transmission-electron-microscopy particle counts and virus titration. In a previous study, when ~40–160 times the amount of HA was administered intranasally via an inactivated virus vaccine, a serum HI antibody response was observed in only 5%–48% of individuals [15]. Moreover, if the antibody response to vaccine in the present study had been induced by nonreplicating antigen, then a booster response should have been observed in H9-seropositive individuals, which was not the case. Thus, it is likely that the observed immune responses resulted from replication of H9N2 G9/AA *ca* vaccine virus at a level below the level of detection of the assays used and that they were not related to deposition of nonreplicating virus.

In summary, the present study found that 2 doses of H9N2 G9/AA *ca* vaccine are well tolerated and immunogenic when administered to H9-seronegative individuals, although replication of the vaccine virus is significantly restricted. The results of the present study suggest that development and evaluation of live attenuated influenza A/AA *ca* vaccines containing novel avian HA and NA of pandemic potential should be continued.

Acknowledgments

We thank Philana Liang, Julie McArthur, Barbara DeNearing, Arlene Bloom, Paula Williams-Soro, Susan DiLorenzo, and Kathy Wang for excellent clinical and technical assistance; Romeo Paredes for data management; and Jamell Davis for assistance with manuscript preparation.

Financial support: National Institutes of Health (contract N01-AI-15444 to X.X.X.); Medlmmune; National Institute of Allergy and Infectious Diseases Intramural Program.

References

1. Chen H, Matsuoka Y, Swayne D, et al. Generation and characterization of a cold-adapted influenza A H9N2 reassortant as a live pandemic influenza virus vaccine candidate. Vaccine 2003;21:4430–6. [PubMed: 14505926]
2. Suguitan AL Jr, McAuliffe J, Mills KL, et al. Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. PLoS Med 2006;3:e360. [PubMed: 16968127]
3. Joseph T, McAuliffe J, Lu B, Jin H, Kemble G, Subbarao K. Evaluation of replication and pathogenicity of avian influenza a H7 subtype viruses in a mouse model. J Virol 2007;81:10558–66. [PubMed: 17634234]
4. Stephenson I, Nicholson KG, Gluck R, et al. Safety and antigenicity of whole virus and subunit influenza A/Hong Kong/1073/99 (H9N2) vaccine in healthy adults: phase I randomised trial. Lancet 2003;362:1959–66. [PubMed: 14683655]
5. Clements ML, Belshe RB, King J, et al. Evaluation of bovine, cold-adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees. J Clin Microbiol 1991;29:1175–82. [PubMed: 1650789]
6. Clements ML, O'Donnel S, Levine MM, Chanock RM, Murphy BR. Dose response of A/Alaska/6/77 (H3N2) cold adapted reassortant vaccine virus in adult volunteers: role of local antibody in resistance to infection with vaccine virus. Infect Immun 1983;40:1044. [PubMed: 6852910]
7. Kandun IN, Wibisono H, Sedyaningsih ER, et al. Three Indonesian clusters of H5N1 virus infection in 2005. N Engl J Med 2006;355:2186–94. [PubMed: 17124016]
8. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937–43. [PubMed: 10074505]
9. Atmar RL, Keitel WA, Patel SM, et al. Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9N2 vaccine preparations. Clin Infect Dis 2006;43:1135–42. [PubMed: 17029131]

10. Hehme N, Engelmann H, Kunzel W, Neumeier E, Sanger R. Pandemic preparedness: lessons learnt from H2N2 and H9N2 candidate vaccines. Med Microbiol Immunol 2002;191:203–8. [PubMed: 12458361]
11. Murphy BR, Coelingh K. Principles underlying the development and use of live attenuated cold-adapted influenza A and B virus vaccines. Viral Immunol 2002;15:295–323. [PubMed: 12081014]
12. Subbarao K, Murphy BR, Fauci AS. Development of effective vaccines against pandemic influenza. Immunity 2006;24:5–9. [PubMed: 16413916]
13. Murphy BR, Rennels MB, Douglas RG Jr, et al. Evaluation of influenza A/Hong Kong/123/77 (H1N1) ts-1A2 and cold-adapted recombinant viruses in seronegative adult volunteers. Infect Immun 1980;29:348–55. [PubMed: 7216417]
14. McMichael AJ, Gotch FM, Noble GR, Beare PAS. Cytotoxic T-cell immunity to influenza. N Engl J Med 1983;309:13. [PubMed: 6602294]
15. Atmar RL, Keitel WA, Cate TR, Munoz FM, Ruben F, Couch RB. A dose-response evaluation of inactivated influenza vaccine given intranasally and intramuscularly to healthy young adults. Vaccine 2007;25:5367–73. [PubMed: 17559990]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Clinical and virologic responses to 1 or 2 $10^{7.0}$-$TCID_{50}$ doses of H9N2 reassortant influenza vaccine (A chicken/Hong Kong/G9/97 × A/Ann Arbor/6/60 *ca*)

			Detection of virus in NW											
			By culture			By rRT-PCR		Participants with illness,[d] %						
Category	Total Participants, no.	participants infected[a], %	Participants, %	Duration,[b] mean (SD), days	Peak titer, mean (SD), $\log_{10}$ $TCID_{50}$/mL[c]	Participants, %	Duration,[b] mean (SD), days	Fever	URI	LRI	Cough	OM	SI	HA
H9N2 seropositive[e]														
Dose 1	9	11	0	0	≤0.6	22	1.0 (0.0)	0	0	0	0	0	11	33
Dose 2	3	67	0	0	≤0.6	33	1.0	0	0	0	0	0	33	33
H9N2 seronegative[e]														
Dose 1	41	39	5	1.0 (0.0)	1.0 (0.4)*	37	1.1 (0.2)	2	10	0	2	0	7	24
Dose 2	24	75	0	0	≤0.6	8	1.5 (0.7)	0	0	0	0	0	0	13

NOTE. HA, headache; LRI, lower-respiratory-tract illness; NW, nasal wash; OM, otitis media; rRT-PCR, real-time reverse-transcriptase polymerase chain reaction; SI, systemic illness (defined as chills and/or myalgias in more than 1 muscle group); URI, upper respiratory tract illness (rhinorrhea).

[a] Infection after immunization with the H9N2 G9/AA ca reassortant vaccine was defined as (1) shedding of vaccine virus detected by culture and/or (2) shedding of vaccine virus detected by rRT-PCR, at any time after study day 1 and/or (3) ≥4-fold increase in serum HI or in either neutralizing or H9 IgG antibodies. Participants whose NW specimens were positive by rRT-PCR on study day 1 only but who did not have evidence of infection were not considered to be infected, because this occurred frequently and we could not exclude the possibility that input virus was being detected.

[b] For those individuals whose NW specimens were positive for vaccine virus.

[c] Culture-negative specimens were assigned a titer of $10^{0.6}$ $TCID_{50}$/mL.

[d] Following dose 1, 1 H9-seronegative participant (2%) had conjunctivitis. Following dose 2, 1 H9-seronegative participant (4%) had an episode of epistaxis.

[e] Individuals were considered (on study day 0) to be H9N2 seropositive if their titers of serum HI to the vaccine virus were >1:8 and were considered to be H9N2 seronegative if their titers were ≤1:8. A total of 9 H9N2-seropositive and 41 H9N2-seronegative individuals received dose 1 of vaccine; of these, 3 H9N2-seropositive and 24 H9N2-seronegative individuals received dose 2 of vaccine.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Antibody responses to 1 or 2 $10^{7.0}$-$TCID_{50}$ doses of H9N2 reassortant influenza vaccine (A chicken/Hong Kong/G9/97 × A/Ann Arbor/6/60 *ca*)

Category	Participants, no.	Participants with any antibody response, %	H9 HI antibody titer			Neutralizing-antibody titer[a]			Titer of serum IgG antibody to rH9			Titer of NW IgA antibody to rH9		
			Mean (SD)		Participants with ≥4-fold increase, %	Mean (SD)		Participants with ≥4-fold increase, %	Mean (SD)		Participants with ≥4-fold increase, %	Mean (SD)		Participants with ≥4-fold increase, %
			Before	After		Before	After		Before	After		Before	After	
H9N2 seropositive														
Dose 1	9	33	4.6(0.7)	4.8 (0.8)	0	6.2(1.8)	6.9 (1.3)	11	6.2(1.1)	6.3(1.0)	0	6.3(2.8)	6.7(1.7)	11
Dose 2	3	33	5.3(0.6)	5.3(1.2)	0	7.0(1.5)	8.0(1.5)	0	6.3(1.2)	6.9(1.2)	33	7.3(0.8)	7.8(0.8)	0
Both doses	3	100	4.3(0.6)	5.3(1.2)	33	6.3(2.0)	8.0(1.5)	67	6.3(1.2)	6.9 (1.2)	67	5.3(2.9)	7.8 (0.8)	67
H9N2 seronegative														
Dose 1	41	29	1.9 (0.8)	2.9 (1.4)	29	3.1(1.1)	3.8 (1.5)	24	5.5 (0.9)	5.9 (1.0)	12	5.5 (1.4)	6.1 (1.3)	2
Dose 2	24	58	2.6(1.3)	4.5(1.2)	58	3.7(1.1)	5.3(1.4)	50	6.1(1.0)	6.4(1.1)	13	5.8(1.5)	6.7(1.7)	13
Both doses	24	100	1.8(0.8)	4.5(1.2)	92	3.2(0.7)	5.3(1.4)	79	5.6(1.0)	6.4(1.1)	50	6.2(1.5)	6.7(1.7)	21

NOTE. All antibody titers are expressed as reciprocal $\log_2$ values. Specimens for measurement of serum and nasal-wash antibody were obtained as indicated in the "Participants, materials, and methods" section of the text; data shown are from before and 4–6 weeks after each vaccination. For measurement of serum IgG antibody and nasal-wash IgA antibody by ELISA, recombinant H9 of the G1 subtype was used. Immulon 2 plates were coated (30 ng/well) with recombinant baculovirus-expressed H9 G1 HA (influenza A/HK/1073/99 H9N2; Protein Sciences). The column entitled "Participants with response to both doses" displays the mean reciprocal $\log_2$ value for this subset of individuals before the first vaccination and after the second vaccination. "Percent of individuals with a response to both doses" also includes some individuals with a 2-fold response to each dose, so the percentage of participants with a response to both doses may be greater than the sum of the percentages responding to each dose. HI, hemagglutination inhibition; NW, nasal wash; rH9, recombinant H9.

[a]The microneutralization assay used in the present study differed from that published elsewhere, in the following respects: (1) the source of Madin-Darby canine kidney cells was the Laboratory of Infectious Diseases of the National Institute for Allergy and Infectious Diseases of the National Institutes of Health; (2) the test virus was H9N2 G9/AA ca; (3) because the test virus was ts, incubation was at 32°C rather than 39°C; (4) the no. of cells/well was 2×10^4, rather than 1.5×10^4; and (5) the amount of virus per well was 80 $TCID_{50}$ rather than 100 $TCID_{50}$.