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# An Open Label Phase I Trial of a Live Attenuated H6N1 Influenza Virus Vaccine in Healthy Adults

**Kawsar R. Talaat**[1], **Ruth A. Karron**[1], **Catherine J. Luke**[2], **Bhagvanji Thumar**[1], **Bridget A. McMahon**[1], **Grace L. Chen**[2], **Elaine W. Lamirande**[2], **Hong Jin**[3], **Kathy L. Coelingh**[3], **George Kemble**[3], and **Kanta Subbarao**[2]

[1]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205

[2]Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

[3]MedImmune, Mountain View, CA 94043

## Abstract

**Background—**We describe the results of an open label Phase I trial of a live attenuated H6N1 influenza virus vaccine. (ClinicalTrials.gov Identifier: NCT00734175)

**Methods and Findings—**We evaluated the safety, infectivity, and immunogenicity of two doses of $10^7$ $TCID_{50}$ of the H6N1 Teal HK 97/AA *ca* vaccine, a cold-adapted and temperature sensitive live, attenuated influenza vaccine (LAIV) in healthy seronegative adults.

Twenty-two participants received the first dose of the vaccine, and 18 received the second dose of vaccine 4 weeks later. The vaccine had a safety profile similar to that of other investigational LAIVs bearing avian hemagglutinin (HA) and neuraminidase (NA) genes. The vaccine was highly restricted in replication: two participants had virus detectable by rRT-PCR beyond day 1 after each dose. Antibody responses to the vaccine were also restricted: 43% of participants developed a serum antibody response as measured by any assay: 5% by hemagglutination-inhibition assay, 5% by microneutralization assay, 29% by ELISA for H6 HA-specific IgG and 24% by ELISA for H6 HA specific IgA after either 1 or 2 doses. Following the second dose, vaccine specific IgG and IgA secreting cells as measured by ELISPOT increased from a mean of 0.6 to $9.2/10^6$ PBMCs and from 0.2 to $2.2/10^6$ PBMCs, respectively.

**Conclusion—**The H6N1 LAIV had a safety profile similar to that of LAIV bearing other HA and NA genes, but was highly restricted in replication in healthy seronegative adults. The H6N1 LAIV was also not as immunogenic as the seasonal LAIV.

## Introduction

Influenza A viruses of the H6 subtype are among the most frequently detected influenza viruses in surveillance studies in birds [1-4]. H6 influenza viruses are of the low pathogenicity phenotype in poultry, and in the last decade, outbreaks of H6 influenza

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Correspondence to: Kawsar R. Talaat.

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infection in commercial poultry have been reported in California in 2000-2002 [5] and in South Africa in 2002-2004 [6].

In 1997, 18 individuals in Hong Kong were infected with a highly pathogenic avian H5N1 influenza that was closely related to strains causing outbreaks in birds [7, 8]. Influenza A viruses of the H6 subtype were isolated from birds at the same time [9]. Nucleotide sequence analysis revealed that the internal protein and NA gene segments of the A/teal/Hong Kong/W312/97 (H6N1) virus were highly similar to those of the H5N1 1997 Hong Kong influenza viruses (>98% sequence and amino acid homology for the six internal protein gene segments, and >97% for the NA gene segment) [9]. Findings from phylogenetic analyses of H6 influenza viruses in Southern China suggested that an A/teal/HK/W312/97-like H6N1 virus may be a precursor of the H5N1 1997 Hong Kong viruses [2, 9]. Both the H5N1 and H6N1 viruses share substantial homology in the 6 internal protein genes with an H9N2 virus that was also identified in bird markets in Hong Kong [2, 9]. The propensity of the related H5N1 and H9N2 viruses to cause human infection [10-13] suggests that this specific constellation of internal protein genes may facilitate human infection with these avian viruses. In addition, a recent study has documented that introduction of a multibasic cleavage site into an H6N1 influenza virus in vitro induces a highly pathogenic phenotype [14]. These findings, coupled with the prevalence of H6 influenza viruses in a wide range of domestic and wild birds, have raised concerns regarding the pandemic potential of H6 influenza viruses.

Although there are no reported cases of natural human illness with H6 influenza, serological surveys suggest that infection is possible. A study of people in rural areas of Southern China revealed that up to 13% of the individuals tested in various provinces had antibodies to H6 influenza [4]. A survey of US veterinarians showed that those who had contact with birds were more likely to be seropositive to H6 HA than were controls without bird contact [15].

In a study of experimental infections of human volunteers with avian influenza viruses, Beare and Webster showed that 3 of 11 people experimentally infected with an H6N1 virus had mild symptoms, and the remainder were asymptomatic. Virus was recovered from the nasal washes of 2 individuals on day 3 or 4 following inoculation. Five other participants were infected with an H6N2 virus: one participant had mild symptoms, although none had recoverable virus [16].

In preparation for the next influenza pandemic, a number of strategies to develop pandemic vaccines are underway [17, 18]. Several reassortant or recombinant vaccines containing avian influenza HA and NA genes and the A/Ann Arbor/6/60 internal protein genes have been developed to date, including candidate vaccines for H9N2, H5N1, H7N3 and H2N2 influenza viruses. Clinical trials have shown that all of these potential vaccine strains are highly attenuated but vary in their capacity to induce antibody responses in humans [19-22].

The live attenuated H6N1 Teal HK 97/AA *ca* vaccine was shown to induce cross protective immunity in mice and ferrets in preclinical studies [23]. Here we describe the results of the first clinical trial of a live attenuated H6N1 influenza virus vaccine.

# Methods

## Vaccine virus

H6N1 Teal HK 97/AA *ca* is a cold-adapted, temperature-sensitive LAIV derived from the low pathogenicity wild-type (*wt*) avian influenza virus A/teal/Hong Kong/W312/97 (H6N1) and the A/AA/6/60 *ca* (H2N2) LAIV Master Donor Virus (MDV-A, MedImmune). H6N1

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Teal HK 97/AA *ca* has the HA and NA gene segments from A/teal/HK/W312/97 and the internal protein genes from the MDV-A virus.

The H6N1 Teal HK 97/AA *ca* vaccine virus was manufactured at MedImmune (Mountain View, CA). The pre-Master Virus Seed (pre-MVS) was derived using reverse genetics [19, 21]. The bulk Drug Substance was filled into Accuspray™ Nasal Spray Systems (Becton-Dickinson, Franklin Lakes, NJ) at a volume of 0.2 mL per sprayer. Each sprayer contained $10^7$ $TCID_{50}$ of the vaccine virus. Filled sprayers were stored frozen at −60°C or below.

## Study Population

This Phase I clinical trial was conducted during the fall of 2008 at the Center for Immunization Research (CIR) outpatient clinic and at the CIR isolation unit at the Johns Hopkins Bayview Medical Center as previously described [19, 21]. The clinical protocol was reviewed and approved by the Western Institutional Review Board (WIRB). Informed, written consent was obtained from each participant. Healthy adult men and non-pregnant women between the ages of 18 and 49 years of age were enrolled in the clinical trial if they met eligibility criteria and were willing to remain on the isolation unit for the duration of the inpatient portion of the trial.

## Study design

This study was conducted as an open-label Phase I inpatient trial with all participants receiving vaccine. Participants were screened to establish health status and if eligible, were given 1 or 2 doses of vaccine four weeks apart as a nasal spray using the Accuspray™ device and examined daily while on the inpatient unit by a health care provider (physician or physician's assistant).

The isolation unit, study design, and study procedures have been previously described [19-21]. Participants were discharged from the isolation unit on study day 9 if rRT-PCR assays for influenza virus were negative on study days 7 and 8.

## Isolation, quantitation and identification of the H6N1 virus

Nasal washes were obtained prior to vaccination and then daily from the day of vaccination until the day of discharge. Specimens were tested for the presence of vaccine virus by quantitative viral culture and by rRT-PCR amplification of a portion of the influenza A M2 gene [19-21]. The limit of detection of vaccine virus was $10^{0.6}$ $TCID_{50}$/mL for virus culture and $10^{0.4}$ $TCID_{50}$/mL for rRT-PCR.

## Immunologic Assays

Sera were tested for hemagglutination inhibiting (HI) antibodies to H6N1 virus as previously described [24]. The sera were tested for neutralizing antibodies using a previously described microneutralization (MN) assay [25]. Our test virus was not *wt* H6N1, but rather the vaccine virus, H6N1 Teal HK 97/AA *ca*.

Sera were also tested for IgA and IgG antibody to the H6 HA by ELISA. Immulon 2 plates were coated with 30ng/well of recombinant baculovirus-expressed H6 HA (rH6; from the homologous vaccine virus) in insect cells (Protein Sciences, Meriden, CT), and the ELISA was performed using endpoint titration [24]. Nasal wash specimens were concentrated as previously described and were tested using the same antigen to measure vaccine-specific IgA, expressed as a percent of total IgA [26].

Total and influenza vaccine specific IgG and IgA antibody secreting cells (ASC) were measured using an enzyme-linked immunospot (ELISPOT) assay based on an assay by

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Sasaki [27], modified as previously described [21]. Briefly, our assay differed from the published assay in that the wells were coated with one of the following (1) beta-propiolactone (BPL)-treated H6N1 Teal HK 97/AA *ca* virus diluted to 5000 HAU/mL in Dulbecco's phosphate-buffered saline (D-PBS, Invitrogen); (2) rH6 HA protein diluted to 10 μg/mL in D-PBS; (3) BPL-treated cold adapted A/Ann Arbor/6/60 (H2N2) MDV-A virus stock (MedImmune) diluted to 5000 HAU/mL in Dulbecco's phosphate-buffered saline (D-PBS, Invitrogen) or purified goat anti-human IgA plus IgG plus IgM (Kierkegaard & Perry Laboratories) at a concentration of 5 μg/mL in D-PBS. PBS alone and human CCRF-CEM cells were used as negative controls; human IM9 cells were used as a positive control. Plate images were recorded and counted using ImmunoSpot 4 software. Human IgA ASC were visualized as red spots and IgG ASC were visualized as blue spots.

### Detection of concomitant viral infections

In participants with respiratory symptoms, nasal wash specimens were cultured for the presence of other respiratory viruses [19]. Rhinoviruses and enteroviruses were detected by rRT-PCR [21, 28].

### Data Analysis

Infection after immunization with the H6N1 Teal HK 97/AA *ca* vaccine virus was defined as: 1) shedding of vaccine virus detected by culture and /or 2) shedding of vaccine virus detected by rRT-PCR any time after study day 1 and/or 3) a ≥4-fold rise in serum HI, neutralizing, or H6 IgG or IgA serum antibodies as measured by ELISA. Participants whose nasal washes were rRT-PCR positive on study day 1 but were without other evidence of infection were not considered infected because we could not exclude the possibility that input virus, rather than replicating virus, was being detected. To calculate means, HI antibody, neutralizing antibody and ELISA reciprocal titers were $\log_2$ transformed.

## Results

### Study participants

Fifty-eight potential participants were screened for enrollment in this study. Twenty-two participants received a first dose of vaccine, and 18 of these individuals received a second dose of vaccine 4 weeks later. Fourteen of the 22 participants were male, 18 were Black, 2 Hispanic, and 2 were White.

Of the 4 participants who did not receive the second vaccination, 1 chose not to return for personal reasons, 1 had an upper respiratory tract illness at the time of the second dose and 2 participants had abnormal laboratory values (decreased hemoglobin level or elevated alanine aminotransferase).

### Reactogenicity

No serious adverse events were reported during the trial. A number of minor illnesses were reported. Headache was the most commonly reported symptom after the first dose of vaccine (6 participants). Three participants reported nasal congestion including 1 who had concomitant symptoms of rhinorrhea and myalgia. Vaccine virus was not detected in this participant by either viral culture or rRT-PCR. One participant who complained of congestion (days 7-11) also had submental lymphadenopathy on days 2-4 and had vaccine virus detected by rRT-PCR on days 1 and 2. The same participant reported sore throat on days 7 through 9, but had no findings of pharyngitis (Table 1). Other illnesses reported following the first dose of vaccine included neck pain (3 individuals) and back pain (1 individual). None of these coincided with vaccine virus recovery or detection, and

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concomitant infection with other respiratory viruses was not detected in by culture or rRT-PCR amplification of RNA from nasal washes obtained from these participants.

Following the second dose of vaccine, 5 individuals had respiratory illness consisting of rhinorrhea (4 individuals), nasal congestion (3 individuals), cough (2 individuals) or wheezing (1 individual). Vaccine virus was detected by rRT-PCR on day 1 from 3 of these participants; however, only 1 participant had symptoms that began on that day (his rhinorrhea lasted until day 6). Interestingly, a participant who had rhinorrhea, cough, nasal congestion, and wheezing on days 1-7 (the cough lasted until day 12) did not have vaccine virus detected by culture or rRT-PCR, but was positive for parainfluenza 3 (PIV3) on day -1. An additional participant who complained of headache, rhinorrhea, nasal congestion, and malaise was positive for PIV3 on days 4 through 6 (concomitant with symptoms), but also had vaccine virus recovered by rRT-PCR on day 1. One participant was positive for rhinovirus on days 1 and 2 and had concomitant complaints of rhinorrhea, nasal congestion, and fatigue. There were five complaints of headache among 3 participants (Table 1). Other minor illnesses after the second dose included dizziness (1 individual), muscle soreness (2 individuals), abdominal pain (1 individual), and abdominal distension (1 individual). One participant experienced submental lymphadenopathy on days 3 through 5; she had vaccine virus recovered by rRT-PCR on days 1 and 2.

### Vaccine virus replication

The H6N1 Teal HK 97/AA *ca* vaccine virus was highly restricted in replication. No participants had vaccine virus recovered by culture from nasal wash after either dose. When the nasal wash specimens were tested by rRT-PCR, vaccine virus was detected on day 1 only (6 individuals) and on days 1 and 2 (2 individuals) after the first dose. After the second dose, vaccine virus was detected by rRT-PCR on day 1 only (4 individuals), on days 1 and 2 (1 individual), and days 1 and 4 (1 individual) (Table 1).

### Immune Responses

Sera were available for analysis from 21 of 22 participants who received the first dose of vaccine and from all 18 participants who received the second dose of vaccine. One of the participants (5%) had an 8-fold rise in serum HI and neutralizing antibody titers after the first dose of vaccine (Table 2). After the second dose, none of the participants had a 4-fold or greater response detected by either assay.

Serum IgG and IgA and nasal wash IgA antibodies to rH6 HA were measured by ELISA (Table 2). A 4-fold or greater rise in serum IgG against rH6 HA was detected in 4 participants (19%) after the first dose of vaccine, and in 2 participants (11%) after the second dose. Three participants (14%) had a 4-fold or greater response by serum IgA after the first dose of vaccine, and 3 after the second dose (17%) (one of whom also responded to the first dose). Only 1 participant (6%) had a 4-fold or greater rise in nasal wash IgA levels after the second dose (Table 2). Over all, 9 of 21 participants (43%) had an antibody response measurable by any assay after any dose of vaccine. We did not see a correlation between detection of virus by PCR and antibody responses.

IgG and IgA ASCs were measured in response to stimulation with either vaccine virus, rH6 HA protein or the Master Donor Virus (MDV-A). After the first dose of vaccine, an increase in the absolute numbers of IgG or IgA vaccine-specific ASCs (≥5 cell increase/$10^6$ PBMCs) each was observed in 1 participant. A greater response in ASC was seen after the second dose of vaccine. The vaccine specific IgG secreting cells increased from a mean of 0.6 to 9.2 cells/$10^6$ PBMCs [range 0-65 after the dose]; 3 participants had ≥5 cell increase/$10^6$

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PBMCs. The vaccine-specific IgA secreting cells increased from a mean of 0.2 to 2.2 [range 0-16 after the dose]; 2 participants had a ≥5 cell increase/$10^6$ PBMCs.

## Discussion

We have reported the first evaluation in humans of a live attenuated H6N1 influenza virus nasal vaccine. The H6N1 Teal HK 97/AA *ca* vaccine had a safety profile similar to that other investigational LAIVs bearing avian HA and NA genes, but was highly restricted in replication and induced antibody responses in a minority of the study participants. Although several individuals had detectable vaccine virus by rRT-PCR the first day after both the first and second vaccinations, and 2 were rRT-PCR positive beyond the first day, none were positive for the vaccine virus by culture after immunization. This is in contrast with the single challenge study done with an H6N1 *wt* virus, in which 2 of 11 participants had virus recovered from nasal washes on days 3 or 4 after inoculation with $10^6$ $EID_{50}$ (50% egg infectious doses) of the challenge virus [16].

There are a number of potential explanations for the lack of infectivity of this vaccine in humans. First, it may be that the H6 HA cannot readily initiate infection in human nasal epithelial cells, because of its receptor binding preference to sialic acid α2,3-galactose (avian-like receptor), and the absence of laboratory confirmed natural human infections suggests that this may be likely. Second, the combination of the A/Teal/HK/97 H6 HA and the A/AA/6/60 *ca* backbone may attenuate the virus to the point that it is not capable of infecting humans. This is not true for all mammals, as Chen et al. have shown that both the wt and *ca* A/teal/HK/97 influenza viruses replicate in the nasal turbinates of ferrets [23]. The restricted replication observed with this vaccine, however, is consistent with that seen with live attenuated avian influenza H5N1 and H9N2 vaccines [19, 20].

Symptoms experienced by the participants were consistent with those seen after other LAIVs. The majority of symptoms after the second dose were most likely related to concomitant infections with PIV3 and rhinovirus, rather than infection with the vaccine virus itself.

Antibody responses to the H6N1 Teal HK/97/AA *ca* vaccine were consistent with the observed restriction in replication: only 1 participant had a 4-fold or greater rise in antibody titer as measured by HI and MN assays. Despite the absence of naturally occurring H6 influenza disease, humans are clearly capable of mounting immune responses to H6 viruses, as is demonstrated by serological surveys of people exposed to birds [4, 15, 29]. In our study, we found that the ELISA for serum IgG was the most sensitive assay for antibody responses, with 29% of participants positive by this assay. This is in contrast with previous LAIV trials of H5N1 and H7N3 vaccines [20, 21], in which we found the serum IgA ELISA to be the most sensitive assay. The H6N1 LAIV also differs from the H9N2 LAIV that was immunogenic despite its poor replication [19]. It is unlikely that the quantity of antigen contained in the inoculum would be sufficient to stimulate an antibody response in the absence of replication. In general, live attenuated A/AA *ca* influenza vaccines contain a much smaller amount of HA than is used in studies of intranasally administered inactivated influenza vaccines [19, 30, 31].

Unlike the study by Sasaki [27], in which ASC were found to be a sensitive measure of response to influenza vaccine, ASC were not detectable in most participants. 1 participant had a 5 cell or greater rise in vaccine-specific IgG ASC after the first dose; after the second dose of vaccine, only 3 participants had significant rises in vaccine-specific IgG secreting cells, 2 of whom also had rises in vaccine-specific IgA secreting cells. Since the seasonal LAIV is protective even in the absence of measurable serum antibodies, it is possible that

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this and other LAIVs induce immune responses that are not measurable by the current assays. Cell-mediated and local immunity induced by LAIV may also contribute, but that might lead to a protective immune response on challenge with homologous or heterologous viruses. In the absence of significant replication of the vaccine virus, or measurable immune response to this or similar live attenuated avian influenza vaccines, strategies to enhance the infectivity and immunogenicity of live-attenuated vaccines against H6 influenza may be needed. We can investigate whether selected mutations can be introduced into the HA gene that will enhance replication without altering antigenicity, as was done to increase the yield of the live attenuated pandemic 2009 H1N1 vaccine [32].

In conclusion, the H6N1 Teal HK 97/AA c*a* vaccine is highly restricted in replication in H6 seronegative healthy human adults and induces low antibody responses as measured by current assays. Additional H6 vaccines need to be evaluated to determine whether other H6 strains that are more immunogenic can be used in pandemic vaccine planning and development.

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## References

1. Cheung CL, Vijaykrishna D, Smith GJ, Fan XH, Zhang JX, Bahl J, et al. Establishment of influenza A virus (H6N1) in minor poultry species in southern China. J Virol. 2007 Oct; 81(19):10402–12. [PubMed: 17652385]
2. Chin PS, Hoffmann E, Webby R, Webster RG, Guan Y, Peiris M, et al. Molecular evolution of H6 influenza viruses from poultry in Southeastern China: prevalence of H6N1 influenza viruses possessing seven A/Hong Kong/156/97 (H5N1)-like genes in poultry. J Virol. 2002 Jan; 76(2):507–16. [PubMed: 11752141]
3. Munster VJ, Baas C, Lexmond P, Waldenstrom J, Wallensten A, Fransson T, et al. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Pathog. 2007 May 11.3(5):e61. [PubMed: 17500589]
4. Shortridge KF. Pandemic influenza: a zoonosis? Semin Respir Infect. 1992 Mar; 7(1):11–25. [PubMed: 1609163]
5. Woolcock PR, Suarez DL, Kuney D. Low-pathogenicity avian influenza virus (H6N2) in chickens in California, 2000-02. Avian Dis. 2003; 47(3 Suppl):872–81. [PubMed: 14575080]
6. Abolnik C, Bisschop S, Gerdes T, Olivier A, Horner R. Outbreaks of avian influenza H6N2 viruses in chickens arose by a reassortment of H6N8 and H9N2 ostrich viruses. Virus Genes. 2007 Jan; 34(1):37–45. [PubMed: 16927114]
7. Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet. 1998 Feb 14; 351(9101):472–7. [PubMed: 9482438]
8. Osterhaus AD, de Jong JC, Rimmelzwaan GF, Claas EC. H5N1 influenza in Hong Kong: virus characterizations. Vaccine. 2002 May 15.20 2:S82–3. [PubMed: 12110266]
9. Hoffmann E, Stech J, Leneva I, Krauss S, Scholtissek C, Chin PS, et al. Characterization of the influenza A virus gene pool in avian species in southern China: was H6N1 a derivative or a precursor of H5N1? J Virol. 2000 Jul; 74(14):6309–15. [PubMed: 10864640]

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10. Butt KM, Smith GJ, Chen H, Zhang LJ, Leung YH, Xu KM, et al. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. J Clin Microbiol. 2005 Nov; 43(11):5760–7. [PubMed: 16272514]
11. Peiris M, Yuen KY, Leung CW, Chan KH, Ip PL, Lai RW, et al. Human infection with influenza H9N2. Lancet. 1999 Sep 11; 354(9182):916–7. [PubMed: 10489954]
12. Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z, Hayden FG, Nguyen DH, de Jong MD, et al. Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med. 2008 Jan 17; 358(3):261–73. [PubMed: 18199865]
13. Gambotto A, Barratt-Boyes SM, de Jong MD, Neumann G, Kawaoka Y. Human infection with highly pathogenic H5N1 influenza virus. Lancet. 2008 Apr 26; 371(9622):1464–75. [PubMed: 18440429]
14. Munster VJ, Schrauwen EJ, de Wit E, van den Brand JM, Bestebroer TM, Herfst S, et al. Insertion of a multi basic cleavage motif into the hemagglutinin of a low pathogenic avian influenza H6N1 virus induces a highly pathogenic phenotype. J Virol. Jun 2.
15. Myers KP, Setterquist SF, Capuano AW, Gray GC. Infection due to 3 avian influenza subtypes in United States veterinarians. Clin Infect Dis. 2007 Jul 1; 45(1):4–9. [PubMed: 17554693]
16. Beare AS, Webster RG. Replication of avian influenza viruses in humans. Arch Virol. 1991; 119(1-2):37–42. [PubMed: 1863223]
17. Luke CJ, Subbarao K. Vaccines for pandemic influenza. Emerg Infect Dis. 2006 Jan; 12(1):66–72. [PubMed: 16494720]
18. Subbarao K, Joseph T. Scientific barriers to developing vaccines against avian influenza viruses. Nat Rev Immunol. 2007 Apr; 7(4):267–78. [PubMed: 17363960]
19. Karron RA, Callahan K, Luke C, Thumar B, McAuliffe J, Schappell E, et al. A Live Attenuated H9N2 Influenza Vaccine Is Well Tolerated and Immunogenic in Healthy Adults. J Infect Dis. 2009 Mar 1; 199(5):711–6. [PubMed: 19210163]
20. Karron RA, Talaat K, Luke C, Callahan K, Thumar B, Dilorenzo S, et al. Evaluation of two live attenuated cold-adapted H5N1 influenza virus vaccines in healthy adults. Vaccine. 2009 Aug 6; 27(36):4953–60. [PubMed: 19540952]
21. Talaat KR, Karron RA, Callahan KA, Luke CJ, DiLorenzo SC, Chen GL, et al. A live attenuated H7N3 influenza virus vaccine is well tolerated and immunogenic in a Phase I trial in healthy adults. Vaccine. 2009 Jun 8; 27(28):3744–53. [PubMed: 19464558]
22. WHO. Tables on the Clinical trials of pandemic influenza prototype vaccines. 2009. 8/25/2009 [cited 2/23/2010]; Available from: http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html
23. Chen Z, Santos C, Aspelund A, Gillim-Ross L, Jin H, Kemble G, et al. Evaluation of live attenuated influenza a virus h6 vaccines in mice and ferrets. J Virol. 2009 Jan; 83(1):65–72. [PubMed: 18945773]
24. Murphy BR, Tierney EL, Barbour BA, Yolken RH, Alling DW, Holley HP Jr, et al. Use of the enzyme-linked immunosorbent assay to detect serum antibody responses of volunteers who received attenuated influenza A virus vaccines. Infect Immun. 1980 Aug; 29(2):342–7. [PubMed: 7011972]
25. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, 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 Apr; 37(4):937–43. [PubMed: 10074505]
26. 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]
27. Sasaki S, Jaimes MC, Holmes TH, Dekker CL, Mahmood K, Kemble GW, et al. Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. J Virol. 2007 Jan; 81(1):215–28. [PubMed: 17050593]
28. Lai KK, Cook L, Wendt S, Corey L, Jerome KR. Evaluation of real-time PCR versus PCR with liquid-phase hybridization for detection of enterovirus RNA in cerebrospinal fluid. J Clin Microbiol. 2003 Jul; 41(7):3133–41. [PubMed: 12843053]

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29. Gray GC, McCarthy T, Capuano AW, Setterquist SF, Alavanja MC, Lynch CF. Evidence for avian influenza A infections among Iowa's agricultural workers. Influenza Other Respi Viruses. 2008 Mar; 2(2):61–9. [PubMed: 18941621]

30. Samdal HH, Bakke H, Oftung F, Holst J, Haugen IL, Korsvold GE, et al. A non-living nasal influenza vaccine can induce major humoral and cellular immune responses in humans without the need for adjuvants. Hum Vaccin. 2005 Mar-Apr; 1(2):85–90. [PubMed: 17038826]

31. Halperin SA, Smith B, Clarke K, Treanor J, Mabrouk T, Germain M. Phase I, randomized, controlled trial to study the reactogenicity and immunogenicity of a nasal, inactivated trivalent influenza virus vaccine in healthy adults. Hum Vaccin. 2005 Jan-Feb; 1(1):37–42. [PubMed: 17038827]

32. Chen Z, Wang W, Zhou H, Suguitan AL Jr, Shambaugh C, Kim L, et al. Generation of live attenuated novel influenza virus A/California/7/09 (H1N1) vaccines with high yield in embryonated chicken eggs. J Virol. Jan; 84(1):44–51. [PubMed: 19864389]

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## Table 1

**Safety Assessment and Virologic Response to 2 Doses of H6N1 Teal HK 97/AA *ca* Vaccine**

| | | | Virus Detection by Culture in Nasal Wash | | Virus Detection by rRT-PCR in Nasal Wash | | | Symptoms Reported No. with Indicated Illness | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subjects | No. of Subjects | % infected[a] | No. Shedding Virus[b] | Peak Titer mean $log_{10}$ $TCID_{50}$/ml[c] | No. pos. on Day 1 | No. pos. after Day 1 | Duration of shedding mean days[d] | Fever | URI | LRI | Ha | Reactogenicity event[e] |
| First Dose | 22 | 32 | 0 | $^{2}0.6$ | 8 | 2[f] | 1.3 | 0 | 3 | 0 | 6 | 7 |
| Second Dose | 18 | 28 | 0 | $^{2}0.6$ | 6 | 2[g] | 1.7 | 0 | 5[h] | 1 | 3 | 6 |

Abbreviations used are as follows: No.=Number, pos.=positive, Ha=headache; URI= Upper respiratory illness (includes rhinorrhea, nasal congestion, cough, pharyngitis) LRI= Lower respiratory illness (includes pneumonia, wheezing, rhonchi)

[a]Infection is defined as shedding of vaccine virus and/or a four fold or greater rise in serum HI antibody titer, serum neutralizing antibody titer, or in serum H6 HA IgG or IgA titer.

[b]Number shedding virus are those participants who had virus recoverable by culture.

[c]Viral titers are expressed as $log_{10}$ $TCID_{50}$/ml. Peak titers of 0.6 $log_{10}$ $TCID_{50}$/ml were assigned to culture-negative samples.

[d]Mean duration calculated for participants who were rRT-PCR positive.

[e]Reactogenicity events are defined as fever, nasal congestion, rhinorrhea, pharyngitis, cough, otitis media, pneumonia, headache, myalgia, chills, conjunctivitis, wheezing, rhonchi and epistaxis. Transient abnormalities in blood pressure, pulse and respiratory rate are not included here, nor are adverse events that were thought to be unrelated or not likely to be related.

[f]2 participants had virus detected by rRT-PCR days 1 and 2.

[g]1 participant had virus detected by rRT-PCR days 1 and 2, the other on days 1 and 4.

[h]Parainfluenza virus type 3 was detected in nasal washes from 2 participants, and rhinovirus was detected in nasal washes from 1 participant

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## Table 2

**Antibody Responses to 2 Doses of H6N1 Teal HK 97/AA *ca* Vaccine**

| | | | Reciprocal mean (SD) $\log_2$ antibody | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Serum HI | | | Serum Neut | | | Serum H6 HA IgG | | | Serum H6 HA IgA | | | Nasal Wash H6 HA IgA | | |
| Subjects | No. of Subjects[a] | % infected[b] | Pre | Post[c] | % with ≥4-fold rise | Pre | Post[c] | % with ≥4-fold rise | Pre | Post[c] | % with ≥4-fold rise | Pre | Post[c] | % with ≥4-fold rise | Pre | Post[c] | % with ≥4-fold rise |
| First Dose | 21 | 32 | 1.0(0.0) | 1.1(0.7) | 5 | 2.3(0.0) | 2.5(0.7) | 5 | 7.1(1.7) | 7.6(1.6) | 19 | 5.3(0.9) | 5.6(1.2) | 14 | 2.4(0.8) | 2.8(0.8) | 0 |
| Second Dose | 18 | 28 | 1.2(0.7) | 1.3(0.8) | 0 | 2.5(0.7) | 2.7(1.0) | 0 | 7.6(1.6) | 7.9(1.5) | 11 | 5.8(1.3) | 6.1(1.9) | 17 | 2.8(0.9) | 2.7(1.0) | 6 |
| Any Dose | 21 | 43 | 1.0(0.0) | 1.2(0.7) | 5 | 2.3(0.0) | 2.6(0.9) | 5 | 7.1(1.7) | 7.8(1.5) | 29 | 5.3(0.9) | 5.9(1.8) | 24 | 2.4(0.8) | 2.8(1.0) | 5 |

Abbreviations are as follows: SD=standard deviation, HI=hemagglutination-inhibiting, neut=neutralizing, Ab=Antibody, HA=hemagglutinin. H6 HA antibody was measured by ELISA.

[a] Data is available from 21 participants for the first dose of vaccine and from 18 for the second dose of vaccine. One participant who received a single dose of vaccine did not return for follow-up visits.

[b] Infection is defined as shedding of vaccine virus and/or a four fold or greater rise in serum HI antibody titer, serum neutralizing antibody titer, or in serum H6 HA IgG or IgA titer. Those with a gradual rise in antibody titer over the entire study period were designated as infected following dose 2.

[c] Post values drawn on day 26 for dose 1 and day 29 for dose 2.