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Potency, Efficacy, and Antigenic Mapping of H7 Avian Influenza Virus Vaccines Against the 2012 H7N3 Highly Pathogenic Avian Influenza Virus from Mexico

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SUMMARY. In the spring of 2012 an outbreak of H7N3 highly pathogenic (HP) avian influenza virus (AIV) occurred in poultry in Mexico. Vaccination was implemented as a control measure, along with increased biosecurity and surveillance. At that time there was no commercially available H7 AIV vaccine in North America; therefore, a recent H7N3 wild bird isolate of low pathogenicity from Mexico (A/cinnamon teal/Mexico/2817/2006 H7N3) was selected and utilized as the vaccine seed strain. In these studies, the potency and efficacy of this vaccine strain was evaluated in chickens against challenge with the 2012 Jalisco H7N3 HPAIV. Although vaccine doses of 256 and 102 hemagglutinating units (HAU) per bird decreased morbidity and mortality significantly compared to sham vaccinates, a dose of 512 HAU per bird was required to prevent mortality and morbidity completely. Additionally, the efficacy of 11 other H7 AIV vaccines and an antigenic map of hemagglutination inhibition assay data with all the vaccines and challenge viruses were evaluated, both to identify other potential vaccine strains and to characterize the relationship between genetic and antigenic distance with protection against this HPAIV. Several other isolates provided adequate protection against the 2012 Jalisco H7N3 lineage, but antigenic and genetic differences were not clear indicators of protection because the immunogenicity of the vaccine seed strain was also a critical factor.

RESUMEN. Potencia, eficacia y mapeo antigénico de vacunas con el virus de la influenza aviar H7 contra un virus altamente patógeno H7N3 del año 2012 de México.

En la primavera del 2012 se presentó un brote del virus de influenza aviar de alta patogenicidad subtipo H7N3 en la avicultura de México. La vacunación se llevó a cabo como medida de control, junto con el aumento de la bioseguridad y la vigilancia. En ese momento no había una vacuna con el subtipo H7 disponible comercialmente en América del Norte, por lo tanto, se seleccionó un aislamiento reciente de baja patogenicidad subtipo H7N3 aislado de aves silvestres de México (A/Pato colorado/México/2817/2006 H7N3) y se utilizó como cepa semilla para la vacuna. En estos estudios, se evaluó la potencia y la eficacia de esta cepa de vacuna en pollos contra el desafío con el virus de alta patogenicidad H7N3 de Jalisco del 2012. Aunque las dosis vacunales de 256 y 102 unidades de hemaglutinación (HAU) por ave disminuyeron la morbilidad y la mortalidad de manera significativa en comparación con las aves no vacunadas, se requirieron dosis de 512 unidades hemaglutinantes por ave para prevenir la mortalidad y la morbilidad completamente. Además, se evaluó la eficacia de otras 11 vacunas con el subtipo H7 y se evaluó un mapa antigénico con los datos por la prueba de inhibición de la hemaglutinación con todas las vacunas y los virus de desafío, para identificar otras cepas de vacunas potenciales y para caracterizar la relación entre la distancia genética y antigénica con protección contra este virus de alta patogenicidad. Otros aislamientos confirieron una protección adecuada contra un virus del linaje H7N3 2012 de Jalisco, pero las diferencias antigénicas y genéticas no fueron indicadores claros de protección debido a que la inmunogenicidad de la semilla vacunal fue también un factor crítico.

Key words: avian influenza, H7N3, vaccine potency, vaccine efficacy

Abbreviations: AIV = avian influenza virus; CL = cloacal; DPC = days post challenge; ECE = embryonating chicken eggs; EID_{50} = 50% egg infectious; GMT = geometric mean titers; HAU = hemagglutinating units; HI = hemagglutination inhibition; HP = highly pathogenic; LP = low pathogenic; MDT = mean death time; OP = oro-pharyngeal; qrRT-PCR = quantitative real-time reverse-transcription PCR; SEPRL = Southeast Poultry Research Laboratory, USDA-Agricultural Research Service; SPF = specific pathogen free

Highly pathogenic (HP) avian influenza virus (AIV) of the H7N3 subtype (10) first occurred in poultry in the state of Jalisco, Mexico, in June 2012 and was reported to be controlled until additional outbreaks occurred starting in January 2013. Vaccination of poultry was implemented in July 2012; however, no pre-existing licensed vaccines were available for the H7 subtype in North America at the time that the decision to vaccinate was made. Therefore, a recent H7 wild duck isolate from Mexico (A/cinnamon teal/Mexico/2817/2006 [H7N3]) was selected for use as the emergency vaccine seed strain. Initial reports from the field indicated that this vaccine was protective and initial experimental challenge study data for this vaccine seed strain and challenge virus combination was protective in chickens (9). However, research is needed to determine the optimal H7 AIV vaccine seed strain for protection against recent H7N3 HPAIV from Mexico as well as the potency for the A/cinnamon teal/Mexico/2817/2006 (H7N3) vaccine seed strain.

In addition to the vaccine and vaccination needs of this outbreak, a crucial question for vaccination of poultry against HPAIV is how to select the most protective vaccine seed strain in general. Ideally a vaccine should be able to prevent mortality, eliminate or substantially reduce morbidity, and reduce the levels of virus shed by infected birds to levels below the infectious dose to decrease transmission potential. A vaccine strain must also be able to replicate well in an *in vitro* production system. Although reverse genetics can be utilized to improve virus replication *in vitro*, the cost of licensing the technology is prohibitively expensive for agricultural use.

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Table 1. Avian influenza virus isolates selected for evaluation as vaccines against challenge with A/chicken/Jalisco/CPA-12283-12/2012 H7N3 HPAIV in chickens. Uppercase letters following values denote statistical groups ($P < 0.05$) within the same column.

Vaccine	Abbreviation	% aa identity with Ck/Jal/12[A]	Antigenic distance to Ck/Jal/12	% Mortality (no./total)	% Morbidity (no./total)	MDT	GMT[B] of antibody to vaccine prechallenge	GMT of antibody to Ck/Jal/12 prechallenge
Sham		NA		100 (10/10)A	100 (10/10)A	2.3	NA	<8
A/chicken/Jalisco/CPA-12283-12/2012 (H7N3)	Ck/Jal/12	100	0	0 (0/10)B	0 (0/10)B	NA[C]	226.3ACD	226.3A
A/Ruddy Turnstone/NJ/892/2006 (H7N7)	RT/NJ/892	97.3	0.9719	0 (0/10)B	0 (0/10)B	NA	242.5ACD	597.1A
A/chicken/BC/514314-1/2004 (H7N3)	Ck/BC/04	97.9	0.6823	0 (0/10)B	0 (0/10)B	NA	113.1ACD	211.1ACD
A/emu/TX/25414/1995 (H7N2)	Em/TX/95	97.0	1.5058	0 (0/10)B	0 (0/10)B	NA	485.0ACD	211.1AD
A/mallard/MD/423/2001 (H7N3)	Ml/MD/01	97.0	0.9304	0 (0/10)B	0 (0/10)B	NA	259.9 A	367.6A
A/turkey/OR/1971 (H7N3)	Tk/OR/71	92.5	1.1461	0 (0/10)B	0 (0/10)B	NA	113.1 ACD	259.9A
A/turkey/VA/67/2002 (H7N2)	Tk/VA/02	92.0	2.5883	0 (0/10)B	0 (0/10)B	NA	870.9 A	394.0A
A/chicken/Chile/176822/2002 (H7N3)	Ck/Chi/02	84.4	2.5574	90 (9/10)A	100 (10/10)A	4.8	13.5BCD	26.4B
A/turkey/Italy/4580/1999 (H7N1)	Tk/Ita/99	82.3	2.6073	10 (1/10)B	10 (1/10)B	2.0	20.0BCD	25.2B
A/chicken/Victoria/1985 (H7N7)	Ck/Vic/85	80.5	2.6706	40 (4/10)AB	70 (7/10)AC	3.5	29.7BCD	15.9BC
A/chicken/Pakistan/447/1995 (H7N3)	Ck/Pak/95	80.2	0.8316	10 (1/10)B	30 (3/10)BC	2.0	51.9BD	40.0BCD

[A]Percent amino acid identity between the HA1 region of the vaccine and challenge virus based on pairwise alignment using the Lipman-Pearson algorithm. aa = amino acid.
[B]GMT by HI assay.
[C]NA = not applicable.

To help elucidate the relationship between challenge virus and vaccine based on antigenic distance and amino acid identity, we evaluated an additional 11 viruses with greater than 80% amino acid identity to the HA1 region of the 2012 Jalisco H7N3 HPAIV (including a homologous vaccine). These viruses were used as vaccines against challenge with the Jalisco 2012 H7N3 HPAIV, and an antigenic map of these H7 AIV isolates using hemagglutination inhibition assay data was also produced to correlate protection with map position.

MATERIALS AND METHODS

Viruses. The challenge virus, A/chicken/Jalisco/CPA-12283-12/2012 (H7N3) HPAIV (Ck/Jal/12) and the virus used as the official vaccine seed strain in Mexico, A/cinnamon teal/Mexico/2817/2006 (H7N3) low pathogenic (LP) AIV (CT/Mex/06) (4,9). All other isolates were obtained from the repository at Southeast Poultry Research Laboratory, USDA-Agricultural Research Service (SEPRL), Athens, GA.

All isolates were propagated in 9- to 11-day-old specific pathogen free (SPF) embryonating chicken eggs (ECE) by standard procedures (13). Allantoic fluid containing infectious virus was inactivated with 0.1% beta-propiolactone, pH adjusted to 7.0 with sodium bicarbonate (12), and used as an antigen source for vaccines and as the antigen for hemagglutination inhibition (HI) assays.

Vaccine preparation. Oil emulsion, adjuvanted vaccines were prepared with Montanide ISA 70 VG (Seppic, Inc., Fairfield, NJ) in accordance with the manufacturer's instructions. The hemagglutination (HA) titers of the allantoic fluid used to produce the vaccines after beta-propiolactone inactivation were standardized to provide a dose of 512 HA units in 0.5 ml (512 HA units per chicken). Sham vaccine was prepared identically using allantoic fluid from uninfected ECE.

Selection of isolates for use as vaccines. In addition to the CT/Mex/06 isolate being used as the official vaccine in Mexico, numerous H7 subtype AIVs were selected for evaluation based on several criteria. First, all isolates were selected because they could replicate to adequate titers in ECE to be considered for use as vaccine seed strains (final concentration sufficient for a dose of 512 HA units in 0.5 ml with adjuvant). Next, isolates were selected to provide diversity of the HA1 amino acid sequence in order to help elucidate the relationship between protection and genetic/antigenic distance. Amino acid identity between the challenge virus and the vaccine viruses are shown in Table 1. One isolate, A/turkey/Italy/4580/1999 (Tk/Ita/99) H7N1, was selected because it was the most closely related isolate available to an existing H7 vaccine strain (A/Turkey/Italy/9289/2002), which had previously been used in Italy. Finally, a homologous vaccine group (inactivated Ck/Jal/12 HPAIV was administered as a vaccine) to the challenge virus was included.

Efficacy of H7 isolates as vaccines against challenge with A/chicken/Jalisco/2012 H7N3 HPAIV. Three-week-old specific pathogen free (SPF) white leghorn chickens from SEPRL in-house flocks were individually tagged for identification and divided into groups. The chickens were housed in biosafety level 3–enhanced facilities in modified Horsfall isolators ventilated with HEPA (high efficiency particle air) intake and exhaust filters. The chickens had *ad libitum* access to feed and water and were cared for in accordance with the guidelines of the SEPRL institutional animal care and use committee. Vaccine prepared as described was administered to each chicken by the subcutaneous route at the nape of the neck (0.5 ml per bird). At 6 wk of age (3 wk post vaccination), serum was collected from all birds to quantify antibodies to the vaccine and challenge virus by HI assay. Also at 3 wk post vaccination, the chickens were challenged with 10^6 50% egg infectious (EID_{50}) doses in 0.1 ml per bird of Ck/Jal/12 by the intrachoanal route to simulate respiratory transmission. At 2 and 4 days postchallenge (DPC) oro-pharyngeal (OP) and cloacal (CL) swabs were collected from each bird for RNA extraction and quantitative real-time reverse-transcription PCR (qrRT-PCR).

Potency of A/cinnamon teal/Mexico/2817/2006 vaccine against A/chicken/Jalisco/CPA-12283-12/2012 H7N3 challenge in chickens. The official H7 vaccine strain being used in Mexico, CT/Mex/06, was evaluated for potency against Ck/Jal/12 by evaluating protection to

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different doses of the vaccine. The same vaccine preparation was used for each dose and precision syringes were used to administer four doses: 0.5, 0.25, 0.1, and 0.01 ml. All other aspects of the challenge, sample collection and processing were identical to the vaccine-challenge study already described (the experiments were conducted simultaneously).

qrRT-PCR. OP and CL swabs collected at 2 and 4 DPC were processed for qrRT-PCR to determine the titer of virus being shed. RNA was extracted as described by Das *et al.* (7) using a combination of Trizol LS (Invitrogen, Inc., Carlsbad, CA) and the MagMAX 96 AI/ND Viral RNA isolation kit (Ambion, Inc. Austin, TX) with the KingFisher magnetic particle processor (Thermo Scientific, Waltham, MA). qrRT-PCR targeting the influenza M gene (15) was performed using the 7500 FAST Real-time PCR System (Applied Biosystems, Foster City, CA), and the AgPath-ID OneStep RT-PCR kit (Ambion, Inc.) in accordance with the U.S. National Veterinary Services Laboratories protocol AVSOP1521/03. The standard curve for virus quantification was established with RNA extracted from dilutions of the same titrated stock of the virus preparation used to challenge the chickens and was run in duplicate.

HI assay. Sera were tested by the HI assay both to produce the data for antigenic cartography and to evaluate antibody levels in vaccinated birds immediately prior to challenge. The HI assay antigens were prepared by inactivating the allantoic fluid from infected ECE with 0.1% beta-propiolactone and adjusting the pH to 7.0 with sodium bicarbonate. The HI assays were performed in accordance with standard procedures (12). Titers were calculated as the reciprocal of the last HI positive serum dilution. Serum to evaluate prechallenge antibody with HI titers of 8 (2^3) or below were considered negative. HI assays for antigenic cartography data were conducted with one modification: the lowest dilution of serum used was 10 (*vs.* 4 for testing antibody levels).

Antigenic cartography. Antigenic cartography (5,14) was used to visualize the HI data and to create a map of the relationships among the HA proteins of the vaccines and challenge virus. Three additional isolates that were used as vaccines in a previous study with Ck/Jal/12 challenge (9), A/turkey/UT/24721-10/1995 (H7N3) (Tk/UT/95), A/chicken/NY/12273-11/1999 (H7N3) (Ck/NY/99), A/quail/PA/20304/1998 (H7N2) (Qu/PA/98), were also included. The HI assay was performed as already described using isolate specific polyclonal chicken sera collected immediately prior to challenge (3 wk post vaccination) from the chickens in the challenge study described above. Sera were treated with 5% chicken red blood cells for 30 min at ambient temperatures to adsorb nonspecific agglutinins. To construct the antigenic map, an HI titer of 10 was used as the low reactor threshold. Normalization was conducted as described elsewhere (3), and the low rank matrix completion multiple dimensional scaling method was used to generate an antigenic map (2,5,6).

Statistical methods. Differences in the titer of virus shed for each vaccine group were tested for statistical significance with one-way ANOVA, all pair-wise with the Tukey test. If normality failed then Kruskal-Wallis one-way ANOVA on ranks, Dunn's method was used (SigmaPlot 12.0, Systat Software, Richmond, CA). Fisher's exact test was used to compare differences in the proportion of birds in each group which shed virus at detectable levels and to evaluate differences in mortality among groups for significance. A P value of ≤ 0.05 was considered to be significant.

RESULTS

Vaccine challenge study. All sham vaccinated chickens died, with a mean death time (MDT) of 2.3 days. There was no mortality or morbidity in any of the groups that received a vaccine with 92.0% or greater amino acid identity to the challenge virus (Table 1). Different rates of mortality were seen in the groups vaccinated with isolates that had 84.4% or less amino acid identity with the challenge virus. There was 10% mortality in the groups vaccinated with A/chicken/Pakistan/447/1995 (Ck/Pak/95) (MDT = 2 days), and A/turkey/Italy/4580/1999 (Tk/Ita/99) (MDT = 2 days); 40% mortality in the group vaccinated with A/chicken/Victoria/1985 (Ck/Vic/85) (MDT = 3.5 days); and 90% in the group vaccinated with A/chicken/Chile/176822/2002 (Ck/Chi/02) (MDT = 4.8 days). Mortality in the Ck/Chi/02 and Ck/Vic/85 vaccinates was not statistically different from the sham vaccinates (Table 1). Morbidity, characterized by moderate to severe lethargy and/or swollen orbits, was primarily observed with chickens that died or were showing severe clinical disease that required euthanasia. Two additional birds in the Ck/Pak/95 vaccinated group, one bird in the Ck/Chi/02 vaccinated group, and three birds in the Ck/Vic/85 vaccinated group presented with morbidity, recovered, and survived.

Oral and CL virus shed was evaluated by qrRT-PCR at 2 and 4 DPC. At 2 DPC, virus was detected in OP swabs in as few as four (40%) birds in each group or as many as 10 (100%) birds per group (Fig. 1). The virus titers detected in OP swabs at 2 DPC were between $10^{3.3}$ and $10^{5.1}$ EID_{50} per bird (Fig. 1). The groups vaccinated with Ck/Chi/02, Tk/Ita/99, Ck/Vic/85. and Ck/Pak/95 shed similar levels of virus as the sham vaccinates. The other vaccine groups shed significantly lower titers (Fig. 1). At 4 DPC, virus was detected in OP swabs from between two (20%) and nine (100%) birds per group and shed titers ranged between $10^{2.9}$ and $10^{6.2}$ EID_{50} per bird. Groups vaccinated with Ck/Chi/02, Tk/Ita/99, and Ck/Vic/85 shed significantly higher titers than the other vaccine groups. At 2 DPC no virus was detected in CL swabs from any birds vaccinated with Ck/Jal/12, A/ruddy turnstone/NJ/892/2006 (RT/NJ/892), A/chicken/BC/314514-1/2004 (Ck/BC/04), A/emu/TX/25414/1995 (Em/TX/95), A/mallard/MD/423/2001 (Ml/MD/423), or A/turkey/OR/1971 (Tk/OR/71). In the remaining groups, virus was detected in CL swabs from 10% (1/10) to 100% (10/10) of the chickens, with titers ranging from $10^{3.1}$ and $10^{6.5}$ EID_{50} per bird; however, mean titers were not significantly different among the vaccine groups (Fig. 1). At 4 DPC, virus was detected in CL swabs from 20% (2/10) to 100% (9/9) of the remaining birds in each group, with titers ranging from $10^{3.1}$ and $10^{6.6}$ EID_{50} per bird (Fig. 1). Titers of virus detected in CL swabs at 4 DPC were not significantly different among the vaccine groups.

Antibody titers to both the vaccine and the challenge virus were determined by HI assay immediately prior to challenge for each bird. Geometric mean titers (GMTs) to each homologous antigen varied among the vaccines (Table 1) from 13.5 (Ck/Chi/02) to 870.9 (Tk/VA/02), and titers to the challenge virus ranged from 15.9 (Ck/Vic/85) to 597.1 (RT/NJ/892) (Table 1). Titers to both vaccines and challenge virus were substantially lower in all the groups vaccinated with the more distantly related viruses (84.4% amino acid identity or less). There did appear to be an inverse relationship between prechallenge antibody titers and mortality and virus shed titers. Although the exact threshold value is not known, vaccines that induced a GMT of at least 100 to the challenge virus prevented mortality and reduced mean oral shed at 2 and 4 DPC to below 10^4 EID_{50}/ml (Fig. 2).

Potency study. No mortality was observed in the full dose group, one bird died in the 50% dose and 20% dose groups each, and there was 100% mortality in the 2% dose group (Table 2). The MDT was substantially longer than that of the sham vaccinates in the 50% dose group, but there was no difference in MDT between the 20% and 2% dose groups and the sham vaccinates. No morbidity was observed in the full dose group. Clinical disease characterized by lethargy, swollen periorbital tissues, and rarely neurological signs was observed with chickens that died or were euthanized due to the severity of disease. In addition to the birds that died, one surviving bird in the 50% dose group and one surviving bird in the 20% dose group presented with moderate lethargy.

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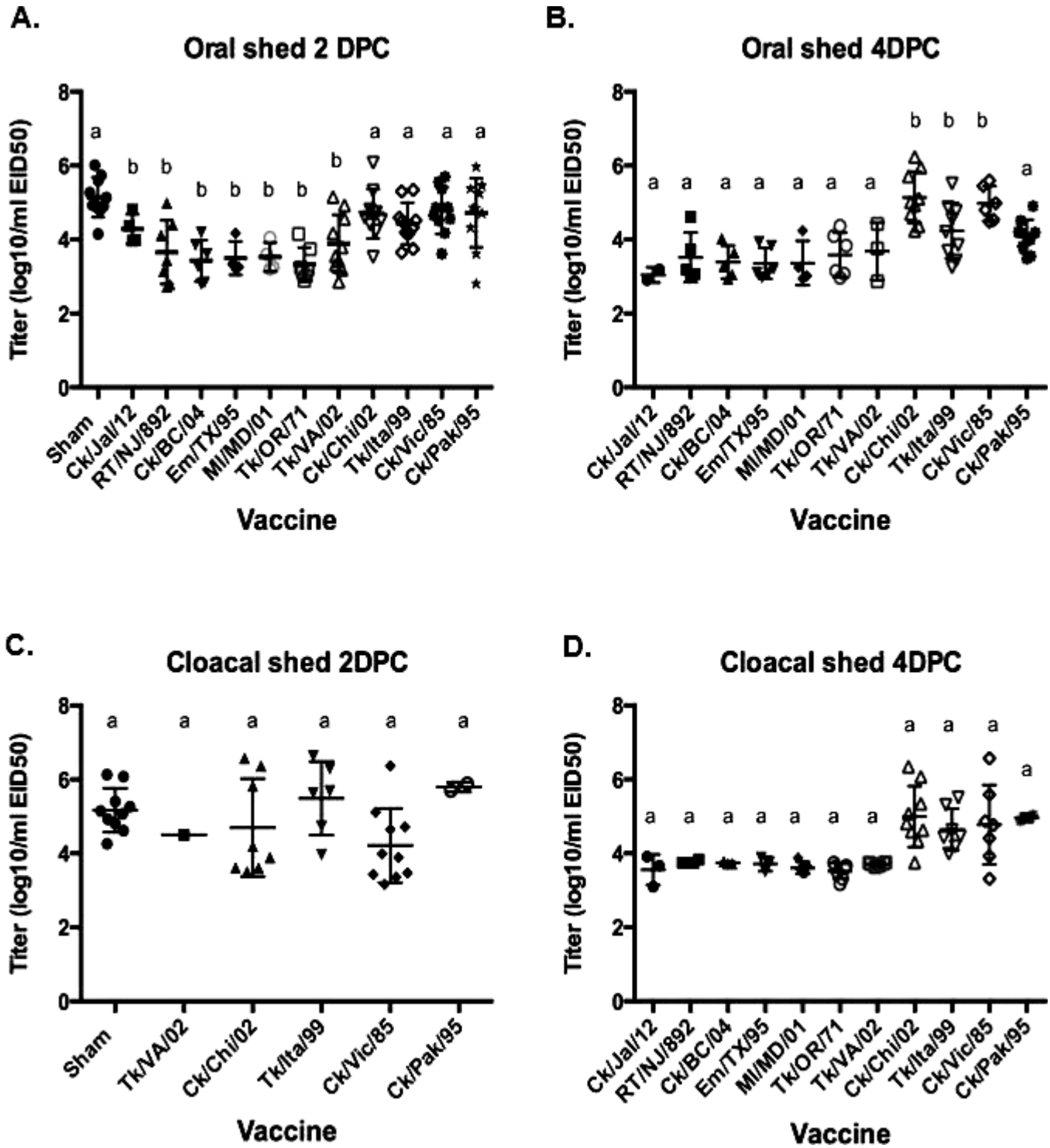

Fig. 1. Titers of virus detected from OP swabs and CL swabs by qrRT-PCR at 2 and 4 DPC for each vaccine: (A) OP swabs collected 2 DPC; (B) OP swabs collected 4 DPC; (C) CL swabs collected 2 DPC; (D) CL swabs collected 4 DPC. Each point represents the titer from an individual animal. The center bar represents the mean and is bracketed by bars representing one standard deviation. Sham vaccine is missing at 4 DPC because all sham vaccinates had died. Vaccines not shown for CL swabs at 2 DPC (Ck/Jal/12, RT/NJ/892, Ck/BC/04, Em/TX/95, Ml/MD/01, TK/OR/71) are missing because there was no virus detected in swab samples from any of these groups in CL swabs 2 DPC. Letters over the data indicated statistical groups for which different letters indicate difference at a P value of <0.05.

Virus was detected with OP and CL swabs from chickens in each of the dose groups at both 2 and 4 DPC; however, the proportion of positives varied from 10% (1/10) of the CL swabs in the full dose group at 2 DPC to 100% positive at both 2 DPC (10/10) and 4 DPC (1/1) with both OP and CL swabs (Fig. 2). Mean titers of virus shed varied among dose groups, and there was a clear trend for lower mean titers to correlate with the higher doses (Fig. 3). Too few birds shed for reliable statistical analysis of CL swabs except between the sham vaccinates and the 2% vaccine dose group at 2 DPC, and there was no significant difference between these two groups. No

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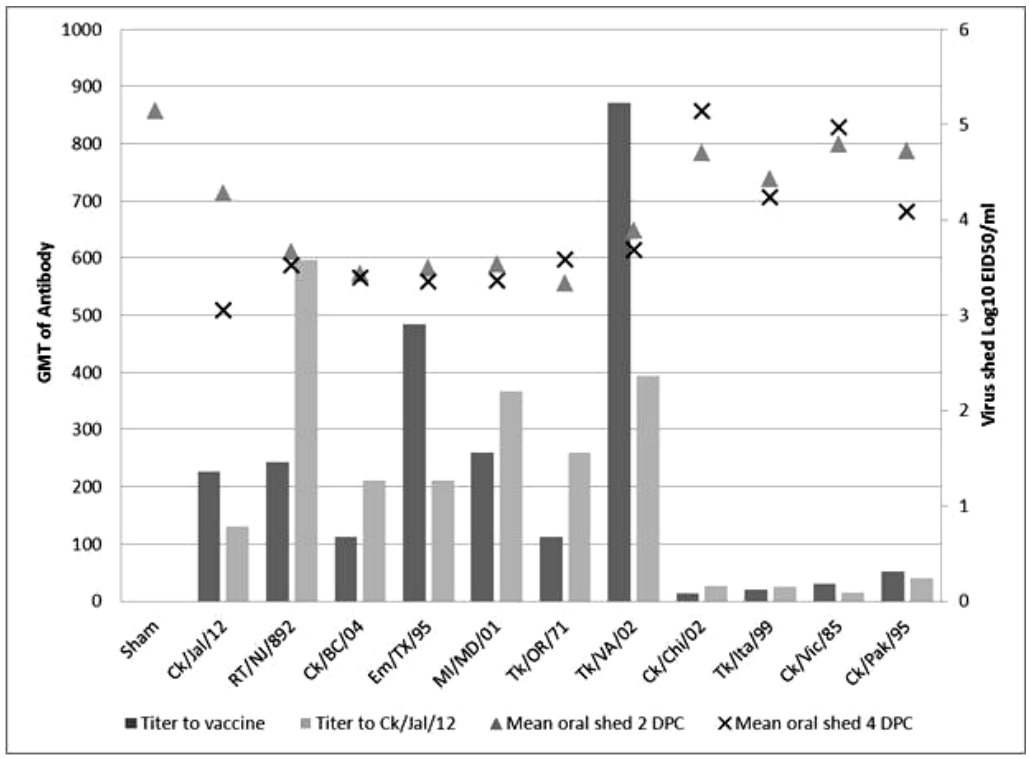

Fig. 2. Antibody titers immediately prior to challenge *vs.* mean virus titers detected from OP swabs by vaccine. Antibody titers to the challenge virus and homologous vaccine (bars, left vertical axis) were determined by hemagglutination inhibition assay 3 wk post vaccination (immediately prior to challenge) and virus titers (lines, right vertical axis) were determined by qrRT-PCR.

sham vaccinates and only one bird was left in the 2% unit group at 4 DPC; therefore, statistical analysis was only conducted with OP swabs from all groups at 2 DPC and the 20%, 50%, and full dose groups at 4 DPC. At 2 DPC, the 20%, 50%, and full dose groups shed significantly less virus orally than the sham or 2% dose group (Fig. 3). At 4 DPC, there was no difference between the 20%, 50%, and full dose groups in OP mean shed titers.

Although the mean antibody titers of the full dose group were higher for both the vaccine and the challenge virus *vs.* the 50% and 20% dose groups prior to challenge (Table 2), the difference was not significant. The chickens in the 2% dose group and sham vaccinates had no detectable antibody to either the vaccine or challenge virus prior to challenge.

Antigenic cartography. The antigenic relationships among the isolates were characterized using HI assay with sera and antigens from all vaccines included in this study and sera from three additional isolates that were used as vaccines in a previous study with the Ck/Jal/12 challenge (9): A/turkey/UT/24721-10/1995 (Tk/UT/95), A/chicken/NY/12273-11/1999 (Ck/NY/99), and A/quail/PA/20304/1998 (Qu/PA/98).

Antigenic cartography showed that the average antigenic distance among these H7 isolates was 1.8731 units. The maximum distance was 3.8917 units (between Ck/Vic/85 and CT/Mex/06), and the minimum distance was 0.3561 units (RT/NJ/892 and Ml/MD/01), 1 unit is equal to a twofold change in HI titer. The antigenic grouping of the H7 isolates does not seem to correlate with their geographic origins (Fig. 4). For example, Ck/Pak/95 is antigenically close to a number of H7 isolates from North America, including Tk/OR/71, RT/NJ/892, Ml/MD/01, Ck/BC/04, and Ck/Jal/12; the antigenic distances among these isolates is less than 1 unit. The Tk/Ita/99, Ck/Vic/85, and Ck/Chi/02 isolates group together. The antigenic distances between CT/Mex/06 and Tk/UT/95, Ck/Jal/12, and CK/NY/99 are 1.3194, 1.5760, and 1.7260 units, respectively, whereas those between CT/Mex/06 and other H7 isolates tested in this study are more than 2 units. Among all isolates from North America, there is antigenic variation of greater than 2 units, and one possible reason is that we purposely included a genetically diverse number of H7 isolates from North America. The Ck/Jal/12 isolate is antigenically most distant to Ck/Vic/85, Tk/Ita/99, Tk/VA/02, and Ck/Chi/02, each of which have antigenic distances of over 2 units.

Table 2. Mortality, morbidity and antibody titers in chickens inoculated with different doses of vaccine prepared with A/cinnamon teal/Mexico/2817/2006 H7N3 low pathogenic avian influenza virus and challenged 3 wk later with A/chicken/Jalisco/CPA-12283-12/2012 H7N3 HPAIV. Uppercase letters following values denote statistical groups ($P < 0.05$) within the same column.

Vaccine HA units per dose	% of full dose	% Mortality (no./total)	% Morbidity (no./total)	MDT	GMT of antibody to vaccine pre-challenge	GMT of antibody to Ck/Jal/12 prechallenge
512	100	0 (0/10)A	0 (0/10)A	NA	298.6A	149.3A
256	50	7.1 (1/14)A	14.3 (2/14)A	6.0	24.4B	28.3A
102	20	8.3 (1/12)A	16.7 (2/12)A	3.0	20.0B	28.3A
10.2	2	100 (12/12)B	100 (12/12)B	3.0	<2C	<2B
0 (sham vaccine)	0	100 (10/10)B	100 (10/10)B	2.3	NA	NA

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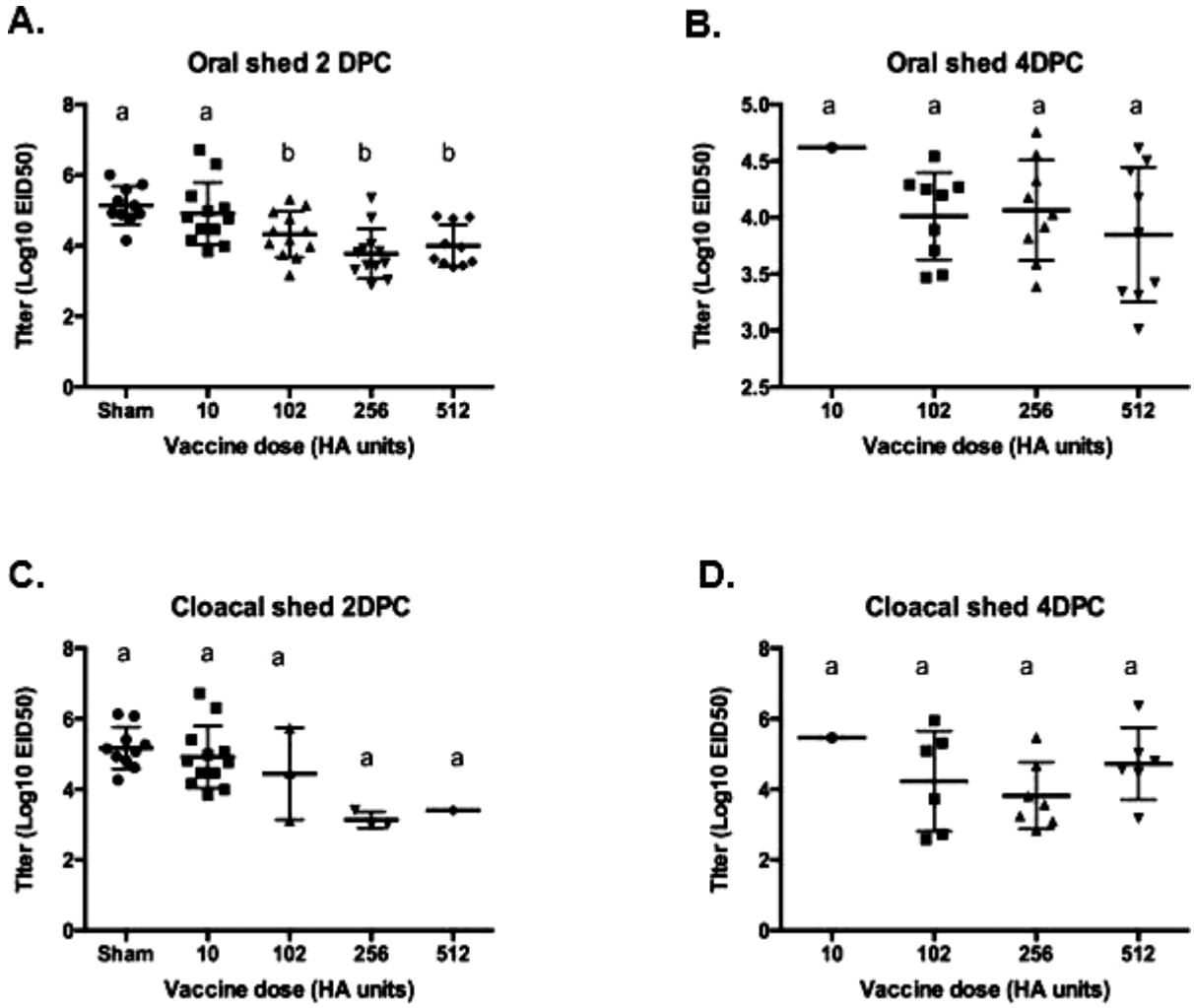

Fig. 3. Titers of virus detected from OP swabs and CL swabs by qrRT-PCR at 2 and 4 DPC for each dose of CT/Mex/06 vaccine: (A) OP swabs collected 2 DPC; (B) OP swabs collected 4 DPC; (C) CL swabs collected 2 DPC; (D) CL swabs collected 4 DPC. Each point represents the titer from an individual animal. The center bar represents the mean and is bracketed by bars representing one standard deviation. Data for sham vaccinate are not shown for 4 DPC because all birds had died. Letters over the data indicated statistical groups for which different letters indicate differences at a P value of <0.05.

DISCUSSION

The vaccine strain used in Mexico to immunize chickens against the Ck/Jal/12 lineage, Dk/Mex/06, was evaluated for potency and was shown to be protective (reducing mortality, morbidity, and shed *vs.* sham vaccinates) at both the full and 50% doses. This indicates that this was a good choice of seed strains for the Ck/Jal/12 virus.

Eleven other H7 viruses were also evaluated for their ability to protect against morbidity and mortality and to reduce virus shed from chickens exposed to Ck/Jal/12 H7N3 HPAIV. Reduction of shed appeared to correspond to the amount of amino acid identity between the vaccine strain and challenge virus. The only vaccines that reduced shed significantly as compared to sham vaccinates were the seven that had 92.0% to 100% (homologous challenge group) amino acid identity in the HA1. Prevention of mortality and morbidity correlated less well. Although all isolates with greater than 92% identity prevented mortality and morbidity completely, the group with the highest mortality was with the vaccine that had 84.4% identity (Ck/Chi/02). Therefore, there were two vaccine groups (Tk/Ita/99 and Ck/Pak/95) with minimal mortality and morbidity that shed comparable levels to the sham vaccinates. Vaccines based on isolates such as these would be poor vaccines for the Ck/Jal/12 lineage because they would mask infection, which complicates surveillance, but would not reduce the level of virus in the environment and therefore could favor silent spread of HPAI virus. Similar relationships, in which the more distantly related a vaccine seed strain is from the challenge virus, the more difficult it is to predict protection, have been described before with HPAIV (1,16,17,18). Until the protective epitopes are identified for H5 and H7 HPAIV, *in vivo* testing will be necessary to fully evaluate vaccinal protection to a given isolate.

In addition to lower sequence identity with the challenge virus than the North American isolates, a confounding issue for the four most distantly related isolates (Ck/Chi/02, Tk/Ita/99, Ck/Pak/95, and Ck/Vic/85) is that they induced low antibody levels. Previous reports with other challenge viruses has shown that vaccines prepared with either Ck/Chi/02 or Ck/Pak/95 induce poor antibody titers (1) and field data supports the inadequate immunogenicity of the H7N3 1995 lineage from Pakistan (11). Therefore, the poor protection provided by these vaccines is likely a combination of genetic/antigenic distance and poor immunogenicity.

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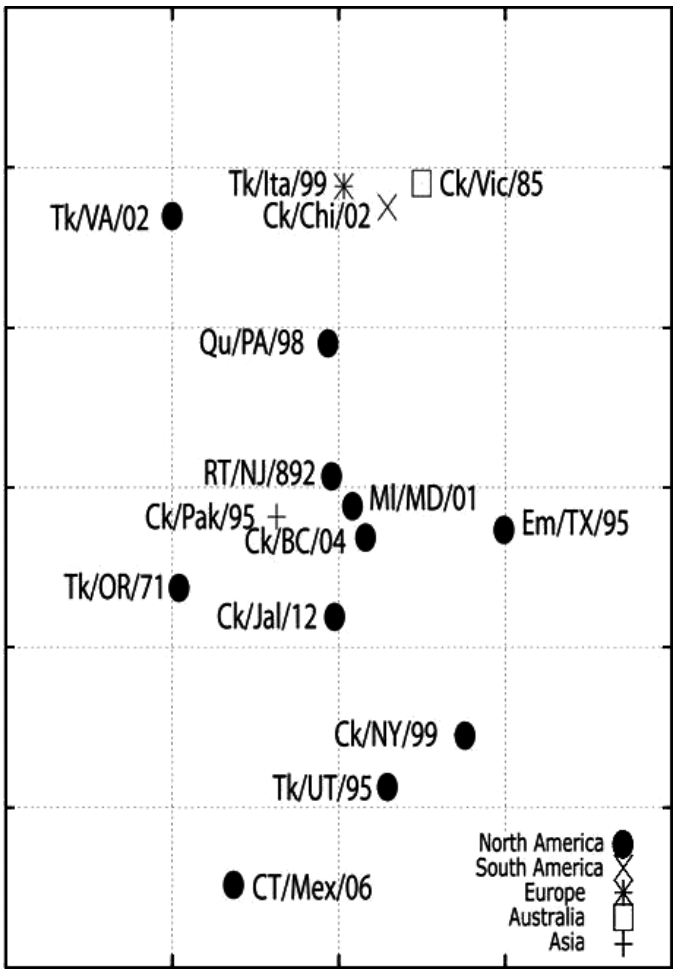

Fig. 4. The antigenic map of HI assay data for H7 avian influenza virus made by AntigenMap (http://sysbio.cvm.msstate.edu/AntigenMap). One grid unit represents a twofold change in HI assay results. The map includes the H7 influenza isolates listed in Table 1.

Based on these data there is no clear predictive value of map distance between a vaccine and challenge virus; the HI data do not directly correlate with protection. For example, there is a small antigenic distance between Ck/Pak/95 and Ck/Jal/12 (0.8316), but Tk/VA/02, which has a greater antigenic distance to Ck/Jal/12 (2.5883), provided better protection against morbidity and mortality. However, as already mentioned there is likely an effect of low antibody titers related to poor immunogenicity of some viruses. Several of the vaccines were also matched to the same subtype of the neuraminidase gene. Previous studies have shown some advantage to high levels of neuraminidase antibody, but the contribution to added protection is minor compared to matched HA antibody (19). Inactivated, oil adjuvanted vaccines are known to primarily stimulate the antibody response with little induction of cell-mediated adaptive immunity, but there could be other aspects of the immune response that are important for protection but are not measured by antibody that inhibits agglutination (e.g., neuraminidase antibody).

The antigenic map also does not correlate well with geographic origin. This has been reported before with H7 AIV (1). It is not clear whether this is a specific characteristic of H7 AIV. Since there has been no sustained vaccination for H7 AIV, pressure to drift from vaccinal immunity would be more limited than for some other influenza subtypes that have different dynamics due to vaccination and maintenance of fewer distinct lineages in a population (e.g., H1, H3, H5, H9 [3,8,14]).

When vaccination is implemented for the control of HPAIV, there is often insufficient time to conduct full efficacy and potency studies of potential vaccine seed strains, and one may be limited to vaccine that is already available for an immediate response. Gene sequence data and HI assay data may be the only information available to help guide that decision. Generally, the most closely related available isolate is the best choice if it is adequately immunogenic and replicates sufficiently well in ECE. In cases in which the available vaccine is not closely related to the challenge virus, protection from mortality and morbidity and reduction of virus shed are difficult to predict because some seed strains are more broadly protective than others due to better immunogenicity and/or induction of broader antibody specificity. Finally, there are numerous factors in the field that contribute to vaccine efficacy, and more importantly, vaccination is only one possible component of a successful of avian influenza control program.

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