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Newcastle disease virus vectors expressing consensus sequence of the H7 HA protein protect broiler chickens and turkeys against highly pathogenic H7N8 virus

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ABSTRACT

Continuous outbreaks of highly pathogenic avian influenza (HPAI) viruses in commercial poultry have caused devastating losses to domestic poultry with a raising public health concern. The outbreaks of HPAI viruses have increased worldwide, including the North America. Therefore, vaccination has been considered as an alternative strategy for an efficient control of HPAI viruses. In this study, we aimed to generate Newcastle disease virus (NDV) vectored H7 serotype-specific vaccines by expressing the consensus sequence of the HA protein. Conventional NDV strain LaSota vector and a chimeric NDV vector containing the avian paramyxovirus type-2 F and HN protein were able to express the consensus sequence of HA protein. The protective efficacy of vaccines was evaluated in broiler chickens and in turkeys. One-day-old poults were prime immunized with the chimeric vector expressing the HA protein followed by boost immunization with LaSota vector expressing the HA protein or co-expressing the HA and NA proteins. Our vaccine candidates provided complete protection of broiler chickens from mortality and shedding of H7N8 HPAI challenge virus. Turkeys were better protected by boosting with the LaSota vector co-expressing the HA and NA proteins than the LaSota vector expressing only the HA protein. Our study demonstrated a potential use of heterologous prime and boost vaccination strategy to protect poultry against H7 HPAI viruses.

1. Background

Highly pathogenic avian influenza (HPAI) virus causes a devastating disease in poultry and is an economically-important pathogen of poultry worldwide. The natural reservoirs of the virus are a wide variety of wild aquatic birds including ducks, gulls and shorebirds [1]. In their natural host, avian influenza viruses typically cause asymptomatic infection and little pathology [2]. In contrast, HPAI virus causes severe disease in terrestrial birds. Avian influenza virus has a genome comprised of eight-segments of negative-sense single-stranded RNA genome and is divided into subtypes on the basis of combination of two viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) (16 HA types and 9NA types) [3]. The HA protein is responsible for virus attachment to the host cell and is the major target of the humoral immune response [4]. The NA protein plays a role in release and spread of progeny virions by removing sialic acid from glycoproteins. The NA antibody has shown to play a role in reducing clinical signs and shedding [5]. Low pathogenic avian influenza (LPAI) viruses contain a HA cleavage site which can only be cleaved by proteases available in intestinal and respiratory tracts [6]. In contrast, HPAI viruses contain multiple basic amino acids at the HA0 cleavage site, resulting in cleavability of HA by ubiquitous intracellular proteases. Therefore, HPAI viruses cause systemic infection and high mortality in chickens and other terrestrial poultry. Two subtypes (H5 and H7) of LPAIVs can naturally change to a highly pathogenic phenotype by acquisition of basic amino acids in the cleavage region of the HA protein through insertion or substitution and recombination with another gene segment(s) or host genome [7,8].

The number of highly pathogenic H7 virus outbreaks has increased in a wide geographic location: H7N1 in poultry farms (Italy); H7N3 in commercial poultry (Australia and Canada) and broiler-breeder chickens (Chile and Pakistan); H7N4 in commercial chickens (Australia); and H7N7 in commercial layer hens (Australia) and poultry (Netherlands, Belgium, Germany Spain)

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[9]. Since 2013, the emergence of novel H7N9 viruses have been a threat to public health by causing severe human infections with high mortality in China [10]. In the U.S., H7N8 HPAI caused outbreaks in turkeys in Indiana in 2016 [11]. The H7N8 virus was eradicated from Indiana after quarantine and depopulation of 414,223 commercial birds on 10 commercial turkey farms. In 2017, H7N9 HPAI was detected in commercial poultry flocks in Tennessee by a spillover of circulating LPAI viruses into commercial poultry [12].

Depopulation of infected flocks is commonly used to control the spread of avian influenza viruses (AIV), including HPAI viruses in poultry. However, the use of vaccines in poultry has increased during the past two decades with the increase in the number of countries with endemic AIVs [13]. Vaccine development has become a critical component not only to control AIV infection in poultry but also to prevent transmission of these viruses from birds to humans. Currently, effective vaccines and efficient vaccination methods are required for a better protection of poultry. Live Newcastle disease virus (NDV) vectored vaccines have shown promising results. However, maternal antibodies to vaccine vectors have hampered the efficacy of NDV vectored vaccines [14]. To overcome this, a chimeric NDV vector (NDV strain Beaudette C) was generated by replacing the ectodomains of F and HN proteins with those of serologically distinct avian paramyxovirus serotype-2 (APMV-2) [15]. The chimeric vector was constructed due to inefficient replication of avirulent APMV-2. The chimeric virus was attenuated to be safe for administration to chickens, but replicated efficiently in vivo. We further established a heterologous prime and boost immunization approach for protection of broiler chickens against H5 HPAI viruses [16,17]. In this study, we generated NDV vectored vaccine candidates against H7 HPAIV. To provide broadly reactive H7 subtype-specific avian influenza vaccine, consensus sequence of the HA protein was expressed by NDV vectors as the protective antigen. Protective efficacy of our vaccine candidates was evaluated in broiler chickens and turkeys, since they have been often involved in the outbreaks of H7 HPAI viruses.

2. Methods

2.1. Generation of chimeric NDV vectored vaccine candidates

To design the HA consensus sequence, HA protein sequences were obtained from the NIAID Influenza Research Database (IRD) (PMID 27679478). Each representative sequence derived from the identical location and time period was selected to eliminate database bias and redundant sequences. A multiple sequence alignment (MSA) was generated using the MAFFT program, version 7 (PMID 23329690). The MSA was then analyzed by a custom Perl program to generate the consensus sequence. The sequence contains the cleavage site sequence of the HA protein of LPAIV (PENPKTR↓GLF). The coding sequence for the HA gene was synthesized with codon optimization since this enhanced the levels of protein expression in our previous studies (16, 17). In addition, co-expression of the HA and NA genes of avian influenza virus enhanced protective efficacy of vaccines. Therefore, the HA and NA genes were cloned in the NDV vectors following our previous cloning approach. Since the HA protein is a major protective antigen, we inserted the HA gene into upstream and the NA gene into downstream of NDV genome by taking an advantage of polar gradient of NDV transcription (16). Briefly, the HA gene was placed between the P and M genes of each NDV vector using the restriction enzyme site of *Pme*I (Fig. 1B). Subsequently, the N8 of NA gene was placed between the M and F genes of NDV strain LaSota using the restriction enzyme site of *Pac*I. Each ORF of the HA and NA genes was flanked by gene-start and gene-end signals of respective NDV vector following the "rule of six." Infectious viruses were generated using NDV reverse genetics following our standard protocol [18]. After the recovery of the vaccine viruses, RT-PCR and sequence analysis were conducted to confirm proper insertion of the HA and NA genes without any mutation.

Expression of the HA and NA proteins in virus-infected chicken embryo fibroblast cell line (DF1) was confirmed by Western blot and immunofluorescence analyses. Each virus was further purified by using a 30% sucrose cushion. Incorporation of the HA and NA proteins into NDV particles was analyzed by Western blot. The efficiency of *in vitro* replication of our vaccine candidates was determined in virus-infected DF1 cells in duplicate [17]. The pathogenicity of these vaccine candidates was determined by intracerebral pathogenicity index (ICPI) test in 1-day-old specific-pathogen free (SPF) chicks. All the animal experiments were conducted following the guidelines and approval of the Animal Care and Use Committee (IACUC) and Institutional Biosecurity Committee (IBC), University of Maryland.

2.2. Protective efficacy of heterologous NDV vectored vaccines in broiler chickens and turkeys

The protective efficacy of our vaccine candidates was evaluated by immunizing 1-day-old broiler chickens (Cornish Cross, Amick Farms Hurlock Hatchery, Hurlock, MD). Twenty chickens were prime immunized with chimeric NDV/HA by the intranasal route (100 μl each, 10^6 pfu/ml).

At 2 weeks post-prime, the chickens were subdivided into two groups (10 chickens for each group) for booster immunization with LaSota/HA or LaSota/HA-NA (200 μl each, 10^6 pfu/ml). One group of chickens remained uninfected as a control group. Prior to boost and challenge experiments, serum samples were collected to monitor the immune responses in the chickens. The antibody titers in serum samples were determined by hemagglutinin inhibition (HI) assay using NDV strain LaSota, chimeric NDV or H7N8 as an individual antigen to evaluate the induction of vector-specific and H7-specific antibody responses. At 2 weeks post-boost, challenge experiment was conducted in our enhanced biosafety level-3 facility. Each group of chickens was intranasally challenged with a lethal dose of H7N8 (A/TY/IN/1403/2016, 10^6 pfu/ml). All the challenged chickens were evaluated on a daily basis for mortality and clinical signs up to 7 days post-challenge (dpc). To monitor the shedding of the challenge virus, oral and cloacal swabs were collected at 3 dpc, inoculated into 9-day-old SPF embryonated chicken eggs, and confirmed by HA assay using chicken erythrocytes.

One-day-old turkeys (bronze turkeys) were obtained from Murray McMurray farm (Hurlock, MD). Similarly, turkeys were prime immunized with chimeric NDV/HA by the intranasal route (100 μl each, 10^6 pfu/ml). In this study, 30 turkeys were prime immunized, since we previously experienced mortality of infected and uninfected turkeys due to an unspecified cause. After 2 weeks, the turkeys were subdivided and boost immunized with LaSota/HA or LaSota/HA-NA (200 μl each, 10^6 pfu/ml). Serological analysis and challenge study were conducted as described above. At 4-weeks post-prime, remaining turkeys (n = 10 for each group) were transferred to enhanced BSL-3 facility for challenge experiment.

2.3. Statistical analysis

Statistically significant differences in serological analysis of different immunized groups were evaluated by one-way analysis of variance (ANOVA) using the Turkey's multiple comparison test. The survival rate was compared using the log-rank test and chi-square statistics. All the results were analyzed by using Prism 5.0

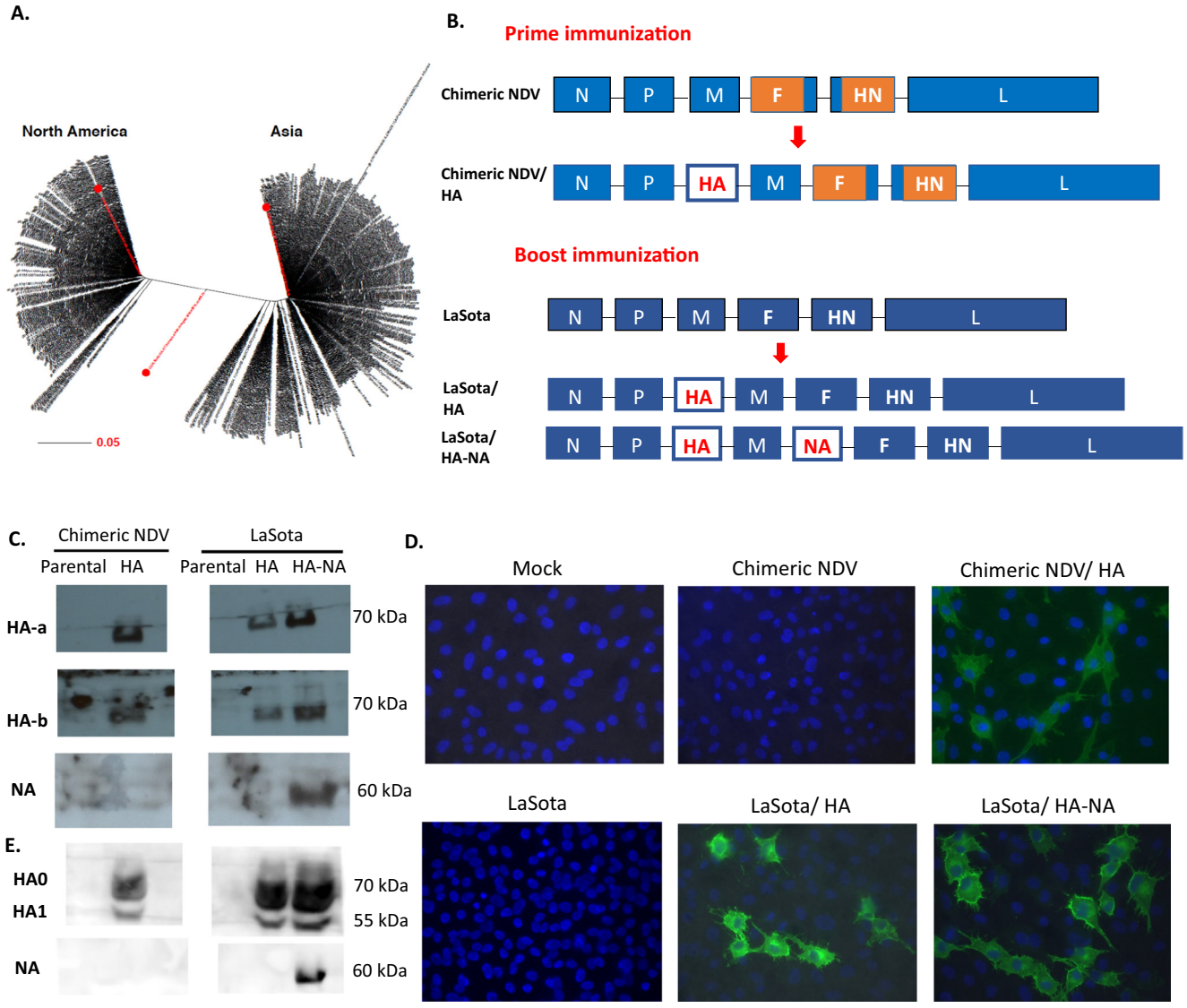

Fig. 1. Generation of chimeric NDV and LaSota vectored vaccine viruses. (A) A phylogenetic tree of the HA sequences of the North American and Asian isolates was generated to determine whether the consensus sequence aligned within the clusters from the original sequences (indicated with red color in North America) (B) The HA and NA genes were placed between the P and M genes and between the M and F genes, respectively. Ectodomains of the F and HN genes derived from APMV-2 are shown as orange rectangle. (C) Expression of H7 HA and N8 NA proteins by NDV vectors were analyzed by Western blot. DF1 cells were infected with each virus at MOI 1. The HA protein in cell lysates was detected by using a monoclonal antibody against H7 HA protein, A/Netherlands/219/2003 (H7N7) (HA-a) and A/Anhui/1/2013 (H7N9) (HA-b). Expression of the NA protein in cell lysates was detected by a polyclonal antibody against N8 NA protein. (D) Surface expression of the HA protein was evaluated by immunofluorescence analysis. DF1 cells were infected with each virus at MOI 1, fixed with methanol at 12 h post-infection, and labeled with anti-HA antibody followed by anti-goat Alexa Fluor 488. (E) Incorporation of the HA and NA proteins into NDV particles was evaluated by Western blot analysis. Parental viruses and vaccine candidates were purified by using a 30% sucrose cushion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(GraphPad Software Inc., San Diego, CA) with a significance level of $P < 0.05$.

3. Results

3.1. Expression of a consensus H7 HA protein by NDV vectors

For induction of a broad protective immunity, we designed a consensus sequence of the HA protein based on the sequences from North American isolates (a total of 186 sequences, dated from 2013 to 2018). The amino acid sequences of the North American HA had 84.8% of identity with those of Asian isolates (n = 334 sequences). A generated phylogenetic tree showed that the consensus sequence aligned with the clusters from the sequences of North American isolates (indicated with red color in North America, Fig. 1A). Further codon optimized HA gene was cloned into two NDV vectors. Chimeric NDV vector containing the HA gene (chimeric NDV/HA) was generated for prime immunization (Fig. 1B). LaSota vector was used to generate boost vaccine candidates: LaSota with HA (LaSota/HA) and LaSota with HA and NA (LaSota/HA-NA).

Western blot analysis confirmed that both the NDV vectors expressed the consensus sequence of HA protein. The HA protein (70 kDa) in DF-1 cell lysates of three vaccine candidates was detected by a monoclonal antibody against H7 HA protein, A/Netherlands/219/2003 (H7N7) (HA-a in Fig. 1C) and A/Anhui/1/2013 (H7N9) (HA-b in Fig. 1C), indicating a broad reactivity of the consensus HA protein. In addition, immunofluorescence analysis showed that all three vaccine candidates expressed the HA protein on the surface of infected DF-1 cells (Fig. 1D). Expression of the NA protein (60 kDa) was specifically detected in

infected DF-1 cells with LaSota/HA-NA using a polyclonal antibody against N8 NA protein (Fig. 1C). Further, expressed HA and NA proteins were incorporated into NDV particles (Fig. 1E). The HA protein was further detected as uncleaved HA0 (70 kDa) and cleaved HA1 protein (55 kDa). Therefore, our comprehensive analyses suggested that the consensus sequence of the HA protein can be a good immunogen.

We determined the *in vitro* replication of vaccine candidates to evaluate whether their replication was affected by the insertion of the HA and NA genes into the genome of NDV (Fig. 2). Our results showed that the chimeric NDV/HA replicated at level comparable to parental chimeric NDV ($p < 0.05$). In contrast, boost vaccine candidates did not replicate efficiently compared to parental LaSota, suggesting that the chimeric NDV vector accommodated the HA gene more effectively than the LaSota vector. Interestingly, LaSota/HA and LaSota/HA-NA replicated to similar levels up to 60 hpi ($p < 0.05$). This indicated that replication of LaSota was not affected by additional insertion of the NA gene into its genome. We further demonstrated avirulence of our vaccine candidates by conducting the ICPI test in 1-day-old SPF chicks. All infected chicks were healthy during the 8 days of infection period (ICPI value of 0.00), thus indicating that they are safe for vaccination of chickens (data not shown).

3.2. Heterologous NDV vectored prime-boost vaccination protected broiler chickens against H7N8 HPAI

Protective efficacy of vaccine candidates was evaluated by prime and boost immunization of broiler chickens. The levels of immunity to NDV in broiler chickens can adversely affect the efficacy of NDV vectored vaccines [17]. In unimmunized chickens, we detected 12.1 and 2.8 HI titers (mean) for LaSota-specific immunity in 2-week-old and 4-week-old broiler chickens, respectively (data not shown). In contrast, HI titers were all negative against chimeric NDV. For vaccination groups, chickens were prime immunized with chimeric NDV/HA and boosted with LaSota/HA (group 1) or LaSota/HA-NA (group 2). In general, the chimeric NDV vector induced similar levels of vector-specific immunity in both groups of chickens after prime immunization (Fig. 3A). In addition, titers of chimeric NDV-specific antibody in chickens did not decrease prior to the challenge, suggesting their lasting immunity in chickens. Boosting with LaSota/HA (group 1) and LaSota/HA-NA (group 2) did not induce high titers of LaSota-specific antibody in 4-week-old chickens (Fig. 3A), indicating that the pre-existing vector immunity in broiler chickens affected replication of LaSota vector. We further evaluated the antibody response to the H7 virus. Chimeric NDV/HA induced >16 HI titers of H7 HA antibodies in

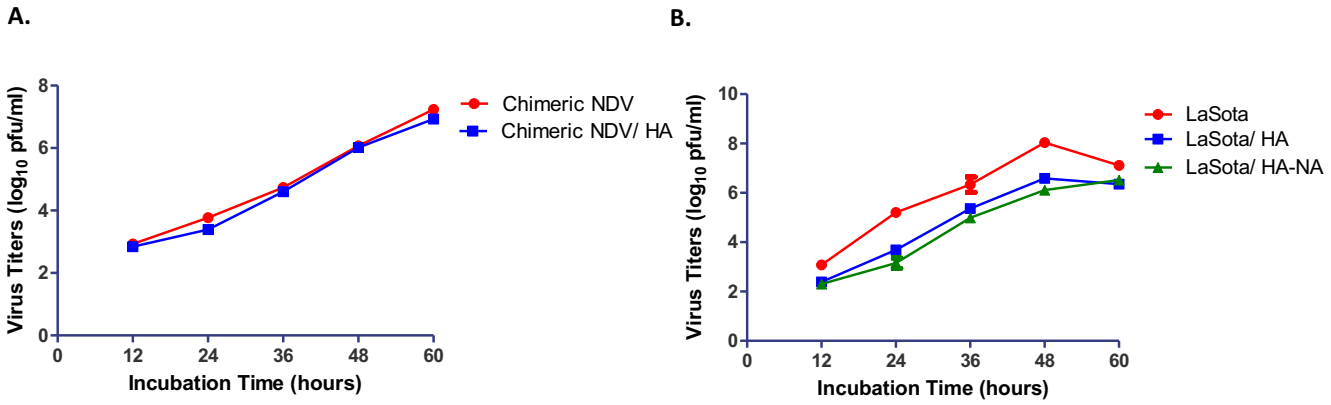

Fig. 2. Characterization of *in vitro* replication of H7 vaccine candidates. The growth kinetics of prime (A) and boost (B) vaccine candidates was determined by infecting DF1 cells with each virus at an MOI of 0.01. Error bars represent the standard deviation.

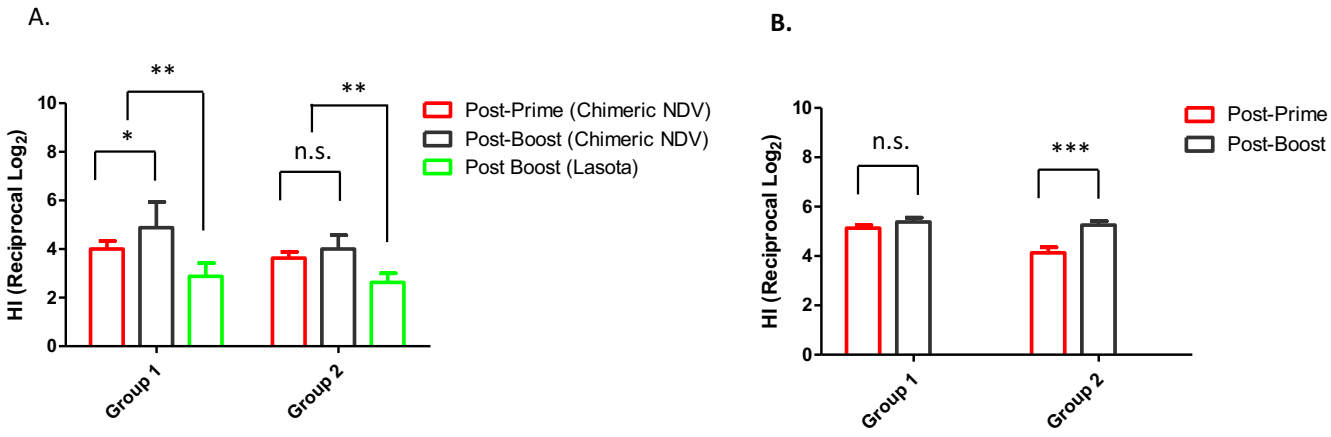

Fig. 3. Immunogenicity of NDV vectored vaccines in broiler chickens. Broiler chickens were intranasally immunized with chimeric NDV/HA (Groups 1 and 2). After 2 weeks, chickens were boosted intranasally with LaSota/HA (Groups 1) or LaSota/HA-NA (Groups 2). Virus-specific antibodies were determined by a hemagglutination inhibition assay using chimeric NDV (prime) and LaSota and chimeric NDV (boost) (A) and H7N8 (B). n.s.: no significant difference. *Significant difference in chimeric NDV-specific immunity between 2-week-old (red bar) and 4-week-old (black bar) broiler chickens ($p < 0.05$). **Significant difference in vector-specific immunity between Chimeric (red and black bars) and LaSota (green bar) vectors ($p < 0.05$). ***Significant difference in H7-specific immunity between post-prime and post-boost chickens ($p < 0.05$). Error bars represent the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

chickens (Groups 1 and 2) at 2 weeks post-prime, suggesting that prime immunization could provide a good level of protective immunity in chickens (Fig. 3B).

Challenge with H7N8 HPAI resulted in 100% of mortality of unimmunized broiler chickens (Fig. 4A). Clinical signs were detected in unimmunized chickens in 2 dpc, and mortality of the chickens was found between 3 and 4 dpc. In contrast, immunized chickens were all survived without exhibiting any clinical signs, and shedding of challenge virus was not detected in chickens (Fig. 4A and B). Therefore, our vaccine candidates provided a complete protection of broiler chickens against mortality, clinical signs, and shedding of H7N8 HPAI.

3.3. Potential use of heterologous NDV vectored vaccination for turkeys against H7N8 HPAI

We then evaluated the protective efficacy of our vaccine candidate in turkeys. One-day-old turkeys were immunized with chimeric NDV/HA followed by boosting with LaSota/HA or LaSota/HA-NA. Similar to chicken immunization, chimeric NDV efficiently induced vector specific immunity in turkeys (HI titers: 16 prior to boost and 64 prior to challenge) (Fig. 5A). We also detected significantly low levels of LaSota vector-specific immunity (HI titer: 4) compared to chimeric NDV-specific immunity after boosting. In H7-specific immunity, prime immunization had induced relatively low levels of HA antibodies, but protective immunity was significantly enhanced in turkeys prior to challenge (Fig. 5B).

At 2 weeks post-boosting, turkeys were challenged with H7N8 HPAI. We found similar patterns in mortality and morbidity between unimmunized chickens and turkeys. H7N8 caused mortality of unimmunized turkeys between 3 and 5 dpc (Fig. 6A). We also found mortality in immunized groups of turkeys (40% and 10% for group 1 and group 2, respectively). Despite this difference in survival rates, one turkey in each group had shedding of challenge virus (Fig. 6B). We had experienced mortality of unvaccinated and vaccinated turkeys during immunization. We were uncertain whether this unspecified cause affected the mortality of immunized turkeys. In general, our findings suggested the potential use of our heterologous vaccination for protection of turkeys against H7N8 HPAI.

4. Discussion

Stamping-out is the main implementation of control strategy for AIV in the U.S [19]. Alternatively, vaccination can be a justifiable tool for control of HPAI when implemented properly and in combination with epidemiological surveillance and biosecurity measures. Vaccination can be used in three ways: as a preventive tool when risk of introduction is high, as an emergency action after

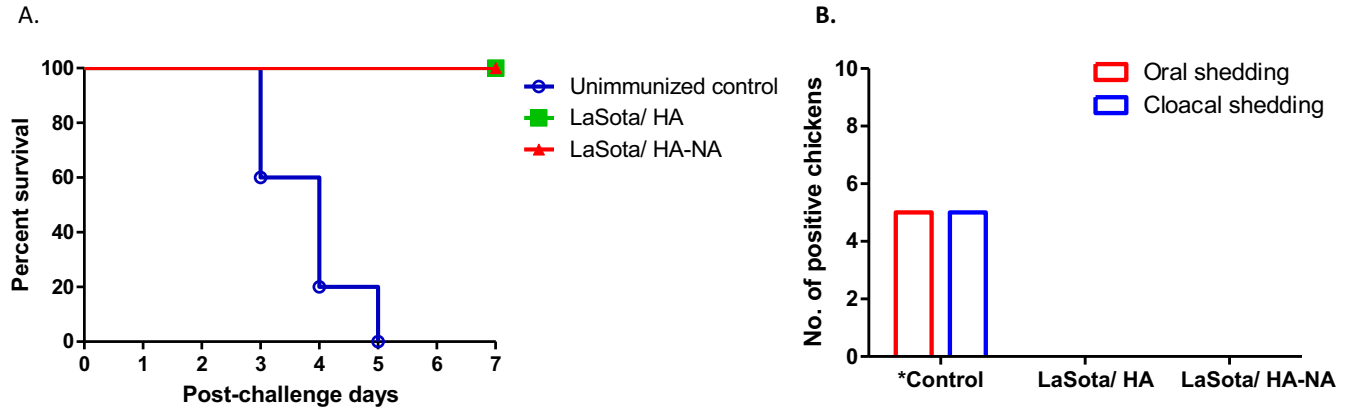

Fig. 4. Protective efficacy of NDV vectored vaccines in chickens. Each immunization group of chickens was challenged with H7N8 HPAIV. Mortality (A) and shedding of challenge virus (B) in broiler chickens were evaluated. *All unimmunized chickens (a total of 5 birds) showed viral shedding.

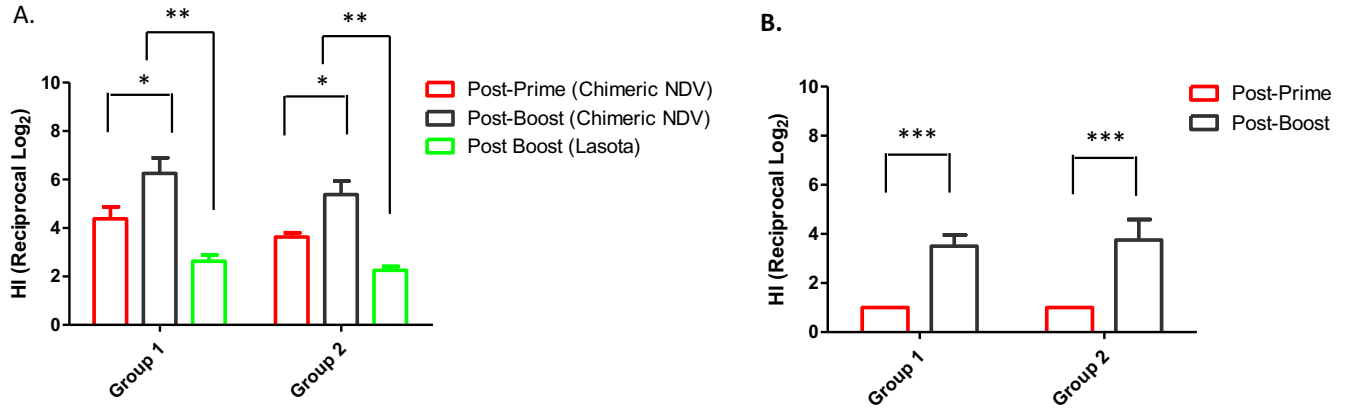

Fig. 5. Immunogenicity of NDV vectored vaccines in turkeys. Each group of turkeys was intranasally immunized with chimeric NDV/HA and then boost immunized with LaSota/HA (Group 1) or LaSota/HA-NA (Group 2). Virus-specific antibodies were determined by a hemagglutination inhibition assay using chimeric NDV (prime) and LaSota and chimeric NDV (boost) (A) and H782 (B). *Significant difference in chimeric NDV-specific immunity between 2-week-old (red bar) and 4-week-old (black bar) broiler chickens ($p < 0.05$). **Significant difference in vector-specific immunity between Chimeric (red and black bars) and LaSota (green bar) vectors ($p < 0.05$). ***Significant difference in H7-specific immunity between post-prime and post-boost chickens ($p < 0.05$). Error bars represent the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

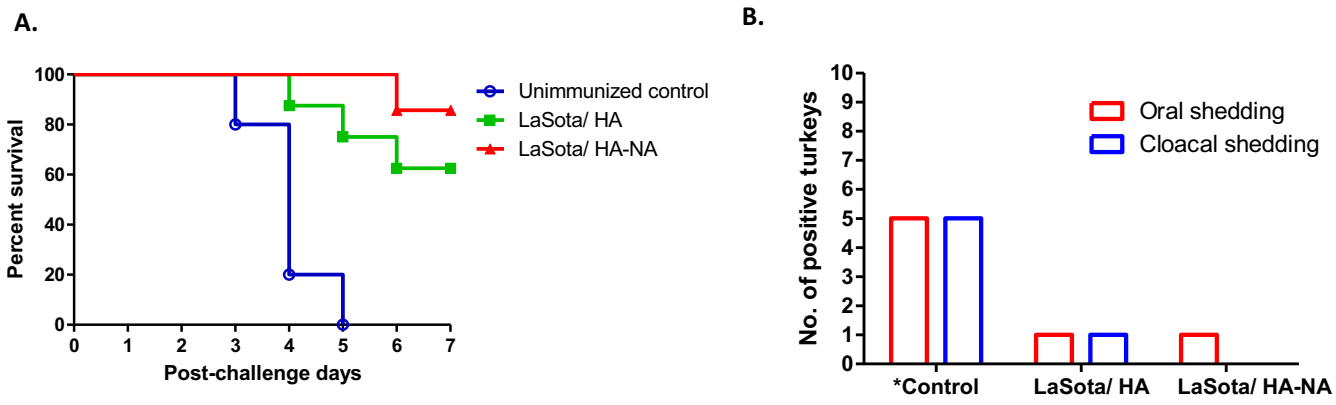

Fig. 6. Protective efficacy of NDV vectored vaccines in turkeys. Prime-boost immunization groups of broiler chickens were challenged intranasally with H7N8 HPAI. Mortality (A) and shedding of challenge virus (B) in turkeys were evaluated. *All unimmunized turkeys (a total of 5 birds) showed viral shedding.

an outbreak begins, or as a routine measure when infection is enzootic within the country (i.e., China, Egypt, Indonesia, and Vietnam) [20,21]. Because avian influenza has a relatively short incubation period, there is insufficient time to prepare a new AI vaccine to control the initial spread of virus. Indeed, the HPAI outbreaks in U.S. commercial poultry flocks have requested for the preparation of available AIV vaccines for the US veterinary stockpile as an additional control plan. H7 subtypes can be classified into two major genetic lineages, the American and Eurasian lineages [9]. H7 viruses are not antigenically diversified as much as H5 viruses (multiple phylogenetic lineages from clade 0 to 9) [22,23]. Therefore, we hypothesized that use of a HA consensus sequence can facilitate preparation of a stockpile for H7 vaccine. To evaluate our hypothesis, we took an advantage of our heterologous NDV vectored vaccination strategy in evaluating.

Previously, conventional NDV vector has shown to induce relatively low levels of H7 HA-specific antibodies in SPF chickens. In H7N7 HPAI challenge study, NDV vectored vaccine provided 90% protection of chickens [24]. NDV vaccine expressing the HA protein of H7N9 A/Anhui/1/2013 was low immunogenic in chickens, and boost and intramuscular immunization were required to enhance humoral immune response in chickens [25]. Challenge with homologous LPAI H7N9 showed protection of chickens from virus shedding. Similarly, a NDV vectored vaccine was generated by expressing the HA protein from a LPAI H7N9 strain [26]. The NDV vectored vaccine elicited undetectable levels of HI and virus neutralization titers but high IgY antibody titers against H7N9 measured by ELISA. The vaccine provided 80% protection of chickens against HPAI H7N9 challenge with reduced viral shedding. Insufficient protective efficacy of NDV vectored H7 vaccines in SPF chickens indicates the requirement of novel vaccine and vaccination approaches to enhance protective immunity. In this study, H7 subtype-specific vaccines were generated with an effective immunization strategy. Consensus sequences of the HA protein have been used for influenza vaccines using various delivery system such as a virus-like particle [27] and a DNA plasmid [28]. Our study also showed that NDV vector can be a good system in expressing the consensus sequence of the HA protein (Fig. 1C–E). Our approach using a replicating viral vector for the delivery is also advantageous in inducing humoral and cellular immune responses.

Viral vectored AI vaccines have been generated by expressing the HA protein as a sole protective antigen [29]. The presence of neutralizing antibodies specific for the HA protein at systemic or mucosal sites of infection provides immediate protection against influenza viruses [30]. In addition, antibodies to the NA protein can impede its receptor-destroying function, thus reducing virus replication by inhibiting virus release from infected cells. Therefore, induction of immunity by co-expression of the two major surface proteins can play an important role in enhancing the protective efficacy of NDV vectored vaccines. In influenza virus, the ratio of HA and NA molecules in the viral envelope usually ranges from 4:1 to 5:1 [31]. We have attempted to recapture this expression ratio by inserting the HA gene into upstream and the NA gene into downstream of NDV vector genome [16,17]. Our studies with H5 HPAI vaccines suggested that co-expression of the HA and NA proteins by chimeric (prime) and LaSota (boost) vectors resulted in a complete protection of broiler chickens against A/Vietnam/1203/2004 H5N1 (clade 1) and H5 clade 2.3.4.4 HPAI viruses. As a proof of concept, we evaluated the protective efficacy of H7-subtype specific vaccines in broiler chickens against H7N8 HPAI. Our heterologous prime and boost immunization led to protection of broiler chickens against mortality and virus shedding. In contrast to our previous H5 vaccine studies, expression of the HA protein by prime and boost vaccine vectors was satisfactory in providing a complete protection of chickens. This needs to be further confirmed by conducting heterologous challenges.

Two-week-old or older SPF chickens have been commonly used to evaluate protective efficacy of NDV vectored AI vaccines [29]. However, their efficacy has been rarely conducted using other domestic poultry. For instance, protective efficacy of NDV expressing LPAI H6 HA was evaluated by immunizing 3-week-old turkeys [32]. Since LPAI H6 virus did not cause mortality and morbidity in challenged turkeys, virus shedding was a major criterion in evaluating the efficacy of vaccine. Shedding in immunized turkeys was marginally reduced compared to control birds. Inefficient protective efficacy may be due to the presence of maternal antibodies to NDV. In this study, the protective efficacy of our vaccine candidates was less efficient in turkeys than in chickens. Our results indicate that heterologous NDV vectors can be potentially used for efficient immunization of turkeys by boosting with LaSota expressing the HA and NA proteins. To enhance the protective efficacy in turkeys, we are planning to evaluate our vaccination by using chimeric NDV and LaSota vectors co-expressing the HA and NA proteins. We have improved our vaccination strategy to facilitate early vaccination of poultry in the field. Our studies with H5 and H7 HPAI vaccines in commercial poultry suggest that NDV vectored vaccines require custom tailored approaches with proper selections of protective antigens, vectors, and vaccination schemes for efficient control of HPAI viruses in the field.

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Declaration of Competing Interests

The authors declare that they have no competing interests.

Contribution

IRC and SGRY conducted the experiments. BGP designed the consensus sequence of the HA protein. SKS and SHK designed the experiments and wrote the manuscript.

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