Newcastle disease virus vectors co-expressing HA and NA proteins protect poultry against highly pathogenic H7N8 avian influenza virus.

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Introduction

- ✓ Highly pathogenic avian influenza (HPAI) viruses cause devastating disease in domestic poultry and are economically-important pathogens worldwide.
- ✓ The HA protein is responsible for virus attachment to the host cell and is the major target of humoral immune response.
- ✓ 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.
- ✓ Disadvantage of currently recombinant NDV-vectored vaccines is their maternal antibodies against poultry.

Objectives

To develop improved recombinant NDV-vectored vaccines against HPAIV in poultry.

Experiments and Results

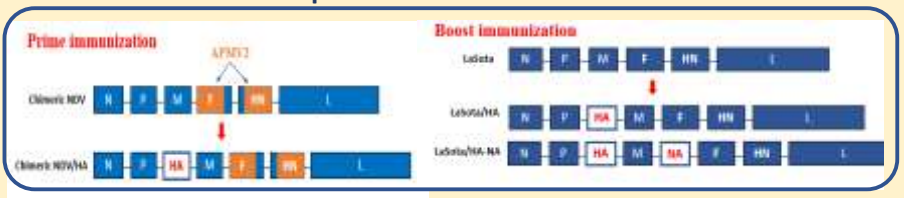
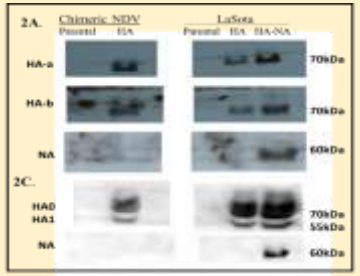


Fig1. Generation of Chimeric and LaSota- vectored vaccine candidates

Infectious viruses were generated using NDV reverse genetics



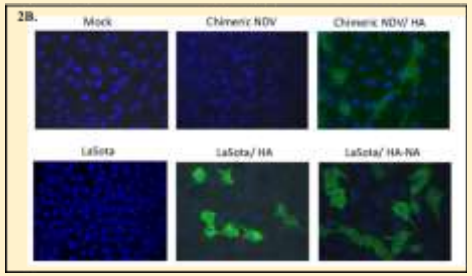


Fig 2A: Western blot: Expression of H7HA and N8NA proteins by NDV vectors. The HA protein in cell lysates was detected by using monoclonal antibodies against H7 HA protein: i)A/Netherlands/219/2003 (H7N7): HA-a, ii)A/Anhui/1/2013 (H7N9): HA-b .Expression of NA protein in cell lysates detected by a polyclonal antibody against N8NA protein. **B**. Immunofluorescence analysis. C. Incorporation of HA and NA proteins into NDV particles purified through 30% sucrose cushion was evaluated by Western blot analysis.

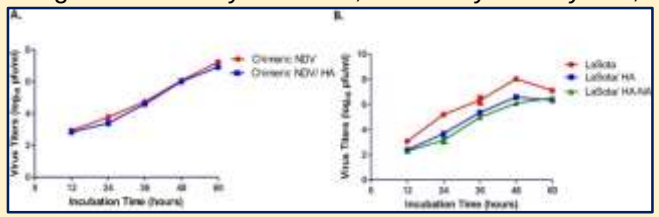


Fig 3. Growth kinetics of prime (A) and boost (B) vaccine candidates .

Animal Experiments

1. Three groups of day-old broiler chicks and turkeys were intranasally injected with 10^5pfu of chimeric NDV/HA for prime immunization. One group was infected with same dose of PBS as control.
2. At day 14,serum samples are collected (post-prime) and they were boost immunized with LaSota/HA and LaSota/HA-NA .
3. After two weeks post-boost, serum samples were collected, and they were challenged with 10^5pfu of HPAIVH7N8(A/TY/IN/1403/2016).
4. Both the broiler chickens and turkeys observed 7 days for clinical signs and mortality.

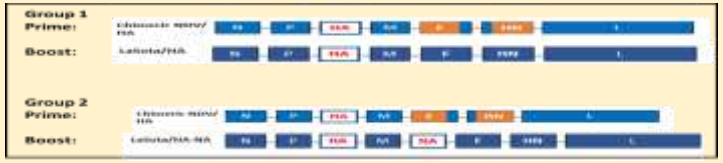


Fig 4: Immunization scheme: Scheme is same in chickens and turkeys. Prime immunization :chimeric NDV/HA for all the chickens and turkeys. 2 weeks post-prime, both chickens and turkeys were divided into two groups for boost immunization: Group1: LaSota/HA, Group 2: LaSota/HA-NA.

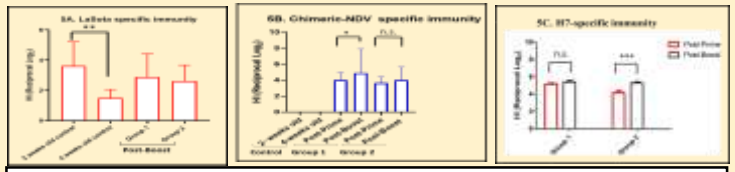


Fig 5. HI assay done for Vector -specific immunity: HI titer of unimmunized chickens at 2 weeks and 4 weeks old age also detected. Here we compared the HI titers of both immunized and unimmunized chickens simultaneously. LaSota- specific immunity (5A, red bar) and chimeric-NDV specific immunity for group 1 and group 2 (5B, blue bar) are showed in respect with control chickens. **Fig.5C: H7- specific immunity measured for both groups of broiler chickens**

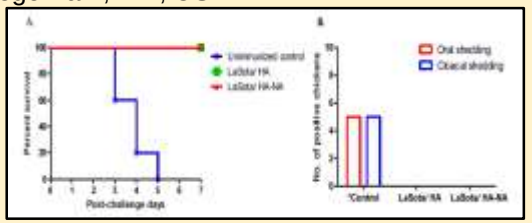
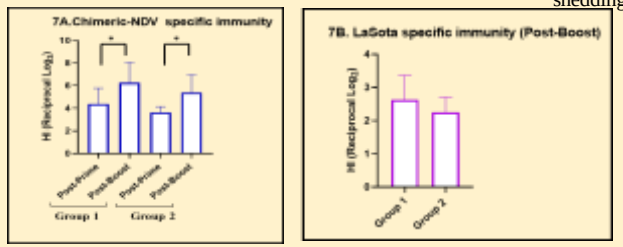


Fig 6: Each immunization group of chickens was challenged with H7N8 HPAIV. Mortality (A) and shedding of challenge virus (B) in broiler chickens were evaluated. *All unimmunized chickens (control, a total of 5 birds) showed viral shedding.



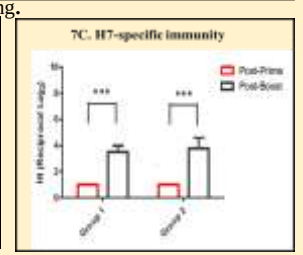


Fig 7. Immunogenicity of NDV vectored vaccines in turkeys. Each group of turkeys was intranasally immunized with chimeric NDV/HA and then boost immunized with LaSota/HA (Group 1) or LaSota/HA-NA (Group 2). Virus-specific antibodies were determined by hemagglutination inhibition assay using chimeric NDV (prime) and LaSota and chimeric NDV (boost) (A) and H7N8 (C). *Significant difference in chimeric NDV-specific immunity between 2-week-old and 4-week-old (blue bar) broiler chickens. ***Significant difference in H7-specific immunity between post-prime and post-boost chickens.

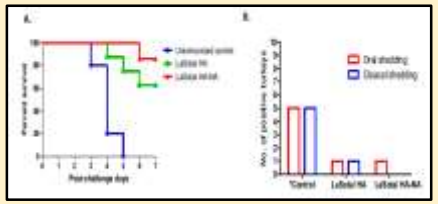


Fig.8. Mortality (A) and shedding of challenge virus (B) in turkeys. All unimmunized turkeys (a total of 5 birds) showed viral shedding.

Conclusions

- ➢ It may possible that vaccination strategy using both chimeric NDV and LaSota vectors coexpressing HA and NA proteins may enhance the protective efficacy in turkeys.
- ➢ NDV vector can be a good system in expressing the consensus sequence of HA protein.
- ➢ In case of broiler chickens, our heterologous prime and boost immunization provided 100% protection from mortality and virus shedding.
- ➢ It may be possible in broiler chickens, only single immunization with our prime vaccine candidate chimeric NDV/HA can give full protection against H7HPAIV.
- ➢ The protective efficacy of our vaccine candidates was less efficient in turkeys.
- ➢ Turkeys were better protected by boosting with the LaSota vector co-expressing the HA and NA proteins (LaSota/HA-NA) than the LaSota vector expressing only the HA protein (LaSota/HA).