Summarized from Avian Diseases, Vol. 55, No. 4, 2011, Pages 667–673

Avian Influenza Adenovirus-Vectored *In Ovo* Vaccination: Target Embryo Tissues and Combination with Marek's Disease Vaccine

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Important Findings

Results suggest that the adenovirus (Ad)-vaccine virus enters the chicken embryo by swallowing and/or through the cloaca. Researchers also found that concurrent *in ovo* vaccination with the experimental adenovirus serotype 5 (AdH5) recombinant vaccine and commercially available Marek's disease virus (MDV) vaccines may cause some interference; avian influenza (AI) vaccine coverage was reduced. However, after increasing the dose of the vectored vaccine the interference was no longer detectable. On the other hand, the results also showed that the Ad-vector vaccination does not interfere with the efficacy of MDV vaccination (Fig. 1).

Significance of Findings

The results provide basic information on how the Ad vector enters the body of the chicken embryo. The results also show that combined vaccination with the Ad vaccine and Mareks disease vaccine is feasible.

Additional Information

Protective immunity against AI can be elicited in chickens by *in ovo* vaccination in a single-dose regimen with a replication competent adenovirus (RCA)-free human Ad serotype 5-vector encoding either the AI virus H5 (AdH5) or H7 hemagglutinins (HAs). *In ovo*-vaccinated chickens are protected against highly pathogenic (HP) AI virus homologous challenges. In addition to *in ovo* injection, ocular administration of Ad expressing the H5 gene has been shown to induce specific immune responses and protection against HPAI virus challenge. Even though *in ovo* vaccination has been routinely performed in the poultry industry for many years, little is known about virus entry sites in the embryo after the injection. This knowledge is of interest in the case of a nonreplicating virus such as the RCA-free Ad vector.

Thus, the researchers investigated embryo tissues targeted by the AdH5-vector when injected into 18-day embryonated eggs. From an applied perspective, it is also relevant to elucidate whether other vaccines routinely delivered by the same route would interfere with Ad-vector vaccination when applied simultaneously, as previously reported for other vaccines. MDV vaccination is routinely performed *in ovo* in the U.S. poultry industry. Theoretically, MDV and Ad-vectored interference could occur as a result of at least three different mechanisms:

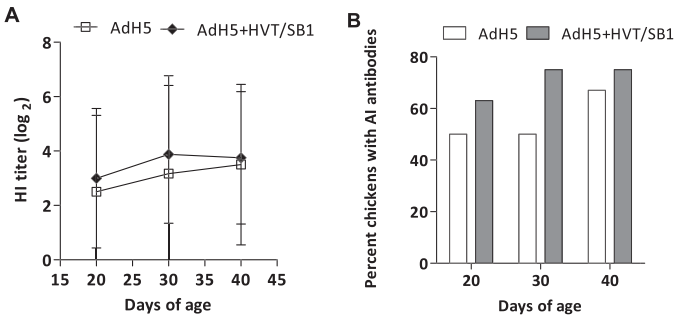

Fig. 1. Chickens were vaccinated with AdTW68.H5ch+HVT/SB-1 (n = 18) or AdTW68.H5ch–only 449 (n = 17). The dose of AdTW68.H5ch vaccine was increased compared to Experiment 1. (A) Avian influenza (AI) H5 hemagglutination inhibition (HI) antibody titers detected in vaccinated chickens. No significant differences ($P > 0.05$) in antibody titers were detected between singly or combined vaccinated groups. (B) AI vaccine coverage (percent chickens with detectable HI antibody titers) in vaccinated chickens. No significant differences ($P > 0.05$) detected between vaccinated groups. HVT/SB-1-only (n = 19) and unvaccinated controls (n = 15) were negative for AI antibodies (not shown).

Fig. 1. Pollos vacunados con AdTW68.H5ch+HVT/SB-1 (n = 18) o AdTW68.H5ch–solamente 449 (n = 17). La dosis de la vacuna AdTW68.H5ch fue aumentada en comparación con el Experimento 1. (A) títulos de anticuerpos contra influenza aviar (AI) H5 por inhibición de la hemoaglutinación (HI) detectados en pollos vacunados. No se detectaron diferencias significativas ($P > 0.05$) en los títulos de anticuerpos entre grupos vacunados con vacuna única o en combinación. (B) cobertura de la vacuna AI (porcentaje de pollitos con título de anticuerpos detectables por HI) en pollitos vacunados. No se detectaron diferencias significativas ($P > 0.05$) entre los grupos vacunados. Tanto el grupo HVT/SB-1-solamente (n = 19) y los que sirvieron como control o testigo sin vacunar (n = 15) fueron negativos a anticuerpos contra AI (no mostrado).

- different replication kinetics (e.g., a fast replicating MDV might induce innate immune responses that would prevent infection by a nonreplicating virus);
- vaccine viruses might compete for the same target cells (e.g., Ads [including the chicken embryo lethal orphan (CELO)] that use coxsackie-Ad receptor receptors for infection expressed by a wide spectrum of cells, including lymphocytes, that are also the target for MDV);
- immunodeficiency caused by MDV (e.g., MDV initially infects B lymphocytes, causing cell depletion, dysfunction, or both and reduced antibody production).

Even though many subsets of T cells can be transformed by MDV, the highest proportion comprises $CD4^+$ T-helper cells expressing a T-cell receptor 2 receptor. Thus, MDV might reduce the immune response elicited by the Ad-vaccine.

 1933-5334 online