Investigating the Efficacy of a Bovine Adenovirus-Vector-Based Vaccine in Poultry Challenged with Avian Influenza Virus

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Background and Significance

The avian influenza virus (AIV) is a major health concern that continues to affect livestock populations and humans. Due to the virus's ability to quickly mutate and spread to non-avian species, researchers are concerned that AIV could have pandemic potential. The AIV reservoir is found in wild aquatic birds as well as bats. Intermediate hosts, such as domesticated chickens, swine, horses, dogs, and turkeys, can transmit the virus to humans.[1]

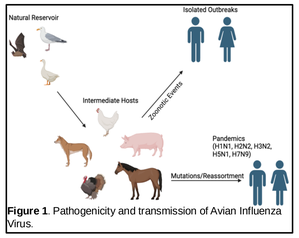


Figure 1. Pathogenicity and transmission of Avian Influenza Virus.

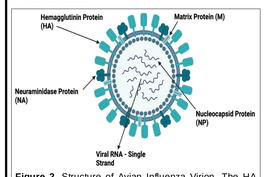


Figure 2. Structure of Avian Influenza Virion. The HA proteins attach to sialic acid residues on glycoproteins to facilitate viral entry. The NA proteins cleave sialic acid residues, allowing the newly formed virus to exit the host cell. The M proteins facilitate viral assembly, and the NP proteins form a protein coat around the viral RNA.

Avian influenza viruses (AIVs) are enveloped, segmented, negative-sense, single-strand RNA viruses that belong to the influenza A virus genus of the *Orthomyxoviridae* family.[2] Their genomes consist of 8 different segments. There are 18 different HA subtypes and 11 different NA subtypes identified in birds.[3] AIVs are also classified into two different groups based on their pathogenicity. Most AIVs fall under the low pathogenicity avian influenza viruses (LPAIV). LPAIVs cause little to no disease in infected poultry. Highly pathogenic avian influenza viruses (HPAIV) have high mortality rates and most often cause severe disease in infected poultry.[4]

Adenoviruses as Vaccine Vectors

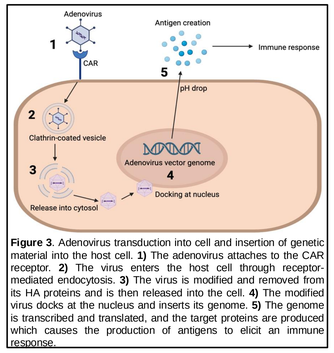


Figure 3. Adenovirus transduction into cell and insertion of genetic material into the host cell. **1)** The adenovirus attaches to the CAR receptor. **2)** The virus enters the host cell through receptor-mediated endocytosis. **3)** The virus is modified and removed from its HA proteins and is then released into the cell. **4)** The modified virus docks at the nucleus and inserts its genome. **5)** The genome is transcribed and translated, and the target proteins are produced which causes the production of antigens to elicit an immune response.

Adenoviruses are double-stranded DNA viruses often asymptomatic but can cause mild respiratory and gastrointestinal infections in humans.

The ability of adenoviruses to induce innate and adaptive immune responses makes them particularly effective as vaccine vectors.[5]

Adenoviral vectors enter the cell through the coxsackievirus-adenovirus receptor (CAR) **(Figure 3)**, present in many different cell types found in the lower respiratory tract, serving as the main replication site for AIVs in humans. The ability of these vaccines to be engineered to target multiple pathogens simultaneously can simplify vaccination schedules and potentially reduce costs.[6]

Bovine Adenovirus Vector

The bovine adenovirus vector is of specific interest due to its high transduction efficiency and enhanced immunogenicity compared to other viral vector options.

Studies have shown that bovine adenovirus vectors, specifically bovine adenovirus type 3 (BAdV3), effectively transduce bovine blood leukocytes, particularly monocytes, and neutrophils, enhancing the potential for immune activation without viral replication.[7]

For instance, a BAdV-3-based influenza vaccine provided complete protection at a dose 30-fold lower than its human counterpart in a mouse model.[8]

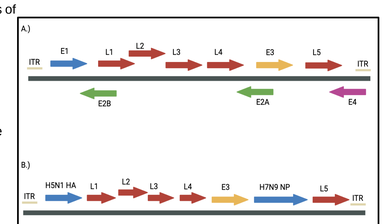


Figure 4. Creation of bovine adenovirus viral vector vaccine. A.) The genome of the unedited bovine adenovirus. B.) Bovine adenovirus H5N1 + H7N9 recombinant vector genome. The E1, E2, and E4 genes were removed to create a replication-deficient viral vector. In place of the E1, E2, and E4 genes, the H5N1 HA gene and the H7N9 Np gene were inserted. This viral vector was grown in cells expressing the E1 gene for replication.

Hypothesis & Research Objectives

- To develop a Bovine adenovirus vector vaccine containing the HA gene of the H5N1 subtype and NP of the H7N9 subtype
- Evaluate the efficacy and optimal dose of this vaccine in three varying vaccine doses ($1x10^6$pfu, $1x10^7$pfu, $1x10^8$pfu) and independent mucosal routes (OR, IN, & IO)
- Evaluate the immunogenicity of the vaccine against H5N2 experimental challenge birds and the efficacy of the vaccine to reduce viral load in the respiratory tracts of challenged birds.

Hypothesis: The Bovine Adenovirus Vector-AIV (BAdV-AIV) vaccine will induce strong mucosal, humoral, and cell-mediated immune responses and significantly reduce the viral load in the respiratory tracts of experimental H5N2 challenge birds.

Fluorescence Activated Cell Sorting (FACS) Gating Strategy

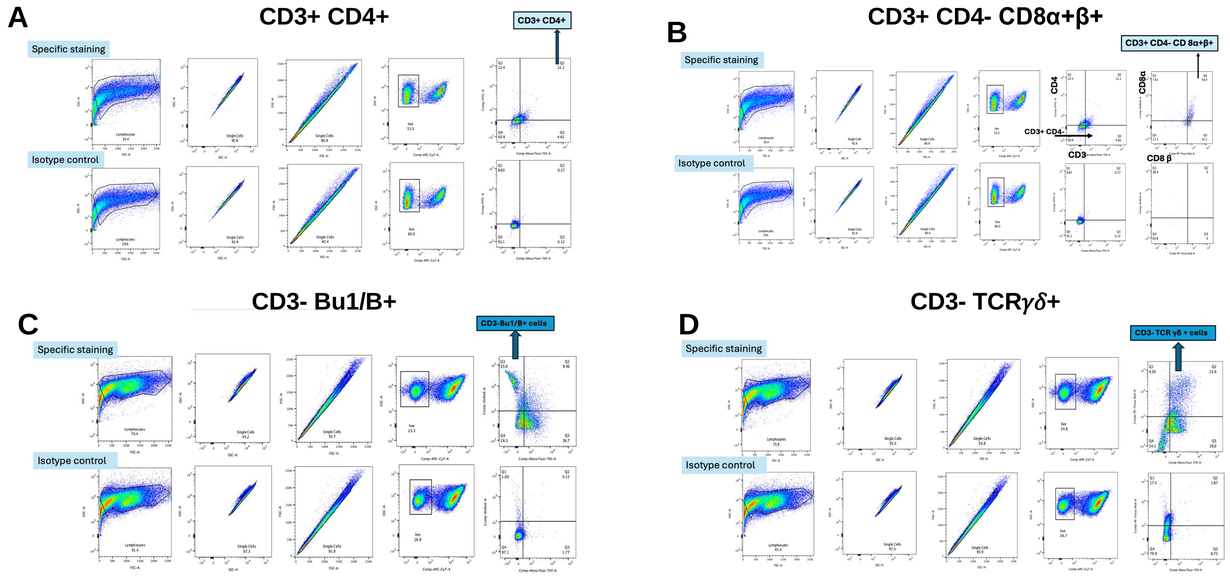


Figure 5. FACS gating strategy. The first four gates of each gating strategy were the same for each cell type. The first gate separated lymphocytes from all other cells collected in the samples using the forward scatter area (FSC-A) against the side scatter area (SSC-A). The second gate separated single cells from cell aggregates and other groups of cells using side scatter height (SSC-H) against SSC-A. The third gating further refines the second gating by re-acquiring all single cells from the second gating; however, this time, forward scatter height (FSC-H) against FSC-A is used. The fourth gating separates live cells from dead cells using the APC-Cy7 area (APC-Cy7-A) against SSC-A. The fifth and sixth gating (if necessary) are specific to cell type. A.) CD3+ CD4+ gating strategy. To separate CD3+CD4+ T-helper cells, cells positive for Alexa-Fluor-700 and FITC were selected. B.) CD3+ CD4- CD8α+β+ gating strategy. To separate CD3+CD4-CD8α+β+ cells, cells positive for Alexa-Fluor-700 and negative for FITC were selected. Then cells positive for PE-Texas-Red and DsRed were selected. C.) Bu-1B- gating strategy. To separate Bu-1B- B-cells, cells positive for Alexa-Fluor-700 and DsRed were selected. D.) CD3+ TCR-γδ+ gating strategy. To separate CD3+ TCR-γδ+ cells, cells positive for Alexa-Fluor-700 and PE-Texas-Red were selected.

Effects of the BAdV-AIV Vaccine on the Cellular and Humoral Immune Response (FACS + HI Assay)

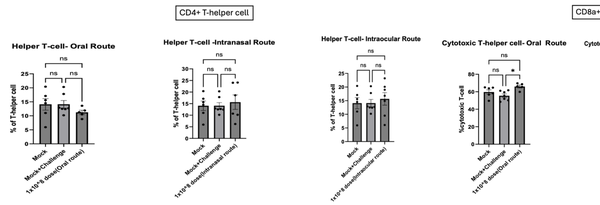


Figure 6. Percentage of CD3+CD4+ T-helper cells from OR, IN, & IO groups from the $1x10^6$ pfu per dose group. No route of vaccination showed significant increases in percentages of CD4+ T-helper cells compared to the mock and mock challenge groups. The intranasal and intraocular groups, however, did show a non-significant increase in CD4+ T-helper cells compared to the mock and mock challenge groups, while the oral route showed a non-significant decrease

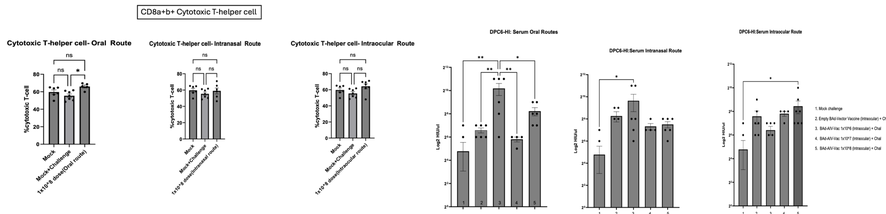


Figure 7. Percentage of CD3+ CD4- CD8α+β Cytotoxic T-helper cells from OR, IN, & IO groups at the $1x10^6$ pfu per dose group. The oral route showed a significant increase in the percentage of Cytotoxic T-helper cells compared to the mock challenge and a non-significant increase compared to the mock group. In both the intranasal and intraocular routes, there was no significant increase compared to the mock challenge and mock groups, however, both routes showed non-significant increases in Cytotoxic T-helper cells compared to both the mock and mock challenge groups.

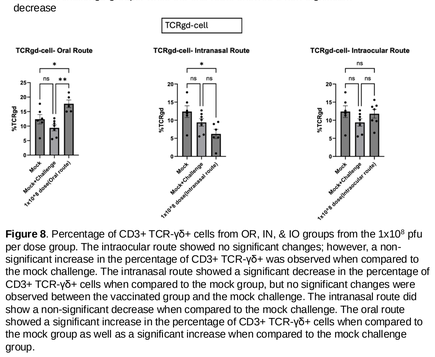


Figure 8. Percentage of CD3+ TCR-γδ+ cells from OR, IN, & IO groups from the $1x10^6$ pfu per dose group. The intraocular route showed no significant changes; however, a non-significant increase in the percentage of CD3+ TCR-γδ+ was observed when compared to the mock challenge. The intranasal route showed a significant decrease in the percentage of CD3+ TCR-γδ+ cells when compared to the mock group, but no significant changes were observed between the vaccinated group and the mock challenge. The intranasal route did show a non-significant decrease when compared to the mock challenge. The oral route showed a significant increase in the percentage of CD3+ TCR-γδ+ cells when compared to the mock group as well as a significant increase when compared to the mock challenge group.

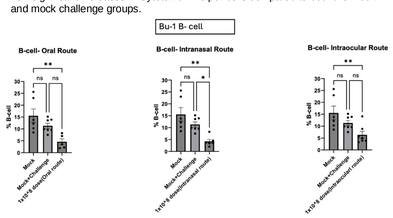


Figure 9. Percentage of Bu-1 B-cells from OR, IN, & IO groups from the $1x10^6$ pfu per dose group. All three routes showed a significant decrease in the percentage of B cells compared to the mock groups. The oral and intraocular groups did not show significant decreases when compared to the mock challenge but did show non-significant decreases in B cell percentages. The intranasal group showed a significant decrease in the percentage of B cells compared to the mock challenge group.

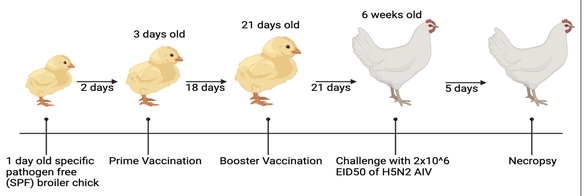
Figure 10. Hemagglutinin-inhibition titers from OR, IN, & IO groups from DPC-6 serum samples. The oral route showed a significant increase in HI units in the $1x10^6$ pfu per dose group as compared to the mock challenge group. The oral route also showed significant decreases in HI titers from the $1x10^7$ – $1x10^8$ pfu per dose group compared to the $1x10^6$ pfu per dose group. The intranasal group also showed a significant increase in HI units in the $1x10^6$ pfu per dose group as compared to the mock challenge group. The intraocular group showed a significant increase in the $1x10^8$ pfu per dose group compared to the mock challenge group.

Conclusions

Overall, these results show that the BAdV-AIV vaccine produced both a humoral and cellular response in broiler chickens challenged with H5N2. The vaccine increased CD4+ T-cell levels and significantly increased CD8+ T-cell populations to create a balanced and robust cellular response. Although the vaccine did not increase B-cell populations, - CD3+TCR-γδ+ cells and antibody levels were significantly increased demonstrating the ability of the vaccine to induce a strong and effective humoral response.

Experimental Design

Group	Vaccine/Doses	Vaccination route
1 (n=6-7)	Mock (Control)	-
2 (n=6-7)	Mock + Challenge (H5N2)	-
3 (n=6-7)	Empty BAd-Vector	Oral
4 (n=6-7)	Empty BAd-Vector	Intranasal
5 (n=6-7)	Empty BAd-Vector	Intraocular
6 (n=6-7)	$1x10^6$pfu/dose BAd-AIV-Vaccine	Oral
7 (n=6-7)	$1x10^6$pfu/dose BAd-AIV-Vaccine	Intranasal
8 (n=6-7)	$1x10^6$pfu/dose BAd-AIV-Vaccine	Intraocular
9 (n=6-7)	$1x10^7$pfu/dose BAd-AIV-Vaccine	Oral
10 (n=6-7)	$1x10^7$pfu/dose BAd-AIV-Vaccine	Intranasal
11 (n=6-7)	$1x10^7$pfu/dose BAd-AIV-Vaccine	Intraocular
12 (n=6-7)	$1x10^8$pfu/dose BAd-AIV-Vaccine	Oral
13 (n=6-7)	$1x10^8$pfu/dose BAd-AIV-Vaccine	Intranasal
14 (n=6-7)	$1x10^8$pfu/dose BAd-AIV-Vaccine	Intraocular



	Days Post Vaccination (DPV) 0 & 21	Days Post Challenge (DPC) 0, 2, & 4	Days Post Challenge (DPC) 6 - Necropsy
Types of Samples Collected	- Cloacal Swab - Oropharyngeal Swab - Blood	- Cloacal Swab - Oropharyngeal Swab - Blood	- Cloacal Swab - Oropharyngeal Swab - Blood - Bile - Small Intestinal Wash - Cecal Tonsil

Future Research

The BAdV-AIV vaccine prototype needs further testing to be able to determine if it can be used as a viable treatment for both chickens and possibly humans in the future. The next step is to test the vaccine against chickens challenged with other heterologous and homologous LPAIVs to determine its ability to produce a cross-protective immune response. Once further tested, the vaccine can be tested against chickens challenged with HPAIVs (H5N1, H7N9) in a BSL3 facility to determine its effectiveness against them as they pose a large threat to domesticated poultry and potentially human populations.

Acknowledgements

To Dr. Gourapura and Comfort at The OSU CFAES: Thank you for all your guidance and support, I could not have done any of this without you and I am thankful for the opportunity I was given to work with you all.
To my family: Thank you for keeping me on track and motivating me to keep working every day.
To Adele: Thank you for being with me every step of the way and giving me the confidence I needed to finish this project.
To my roommates: Thank you for all your help and all the motivation you have given me to put forth my best effort.

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