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Shahid Bahonar University of Kerman

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SHORT COMMUNICATION

Comparative Efficacy of Two Commercial FAdV-4 Vaccines against Virulent Challenge in Broiler Chickens

Arash Ghalyanchi Langeroudi [1], Zahra Ziafati Kafi [1], Soroush Sarmadi [1], Fahimeh Jamiri [1], Alireza Bakhshi [1], Rima Morshed [2], Mahtab Heydarpour [1], Fatemeh Gharagazlou [1], Alireza Vafadar [1], Hirbod Sheikhi [1], Seyed Alireza Rezaee [1], Farnood Shahamati [1], Hossein Hosseini [3*]

[1] *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[2] *Agriculture and Veterinary Group, Faculty of Encyclopedia, IHCS, Tehran, Iran*
[3] *Department of Avian Diseases, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Alborz, Iran*

***Correspondence**
Author's Email:
Hosseini.ho@iau.ac.ir

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Abstract

Hepatitis–hydropericardium syndrome (HHS), caused by fowl adenovirus serotype 4 (FAdV-4), is a major cause of acute mortality in broiler chickens worldwide. Despite the availability of commercial vaccines, their protective efficacy under virulent challenge conditions may differ. This study aimed to compare the immunogenicity and protective efficacy of two commercial FAdV-4 vaccines in broiler chickens. One hundred one-day-old Arian broiler chicks were randomly allocated into four experimental groups (20 birds per group), with five additional contact birds per group to evaluate horizontal transmission. Birds in Groups 3 and 4 were vaccinated at 21 days of age with commercial FAdV-4 vaccines from Company A and Company B, respectively. At 42 days of age, birds in Groups 2, 3, and 4 were challenged intramuscularly with a virulent FAdV-4 strain. Serum samples collected prior to challenge were analyzed for FAdV-4-specific antibodies using an ELISA assay. Birds were monitored for clinical signs, mortality, and gross pathological lesions for seven days post-challenge. Before challenge, birds vaccinated with Company B developed significantly higher ELISA antibody titers (1440 ± SEM) than those vaccinated with Company A (910.5 ± SEM) and unvaccinated controls ($P < 0.0001$). Following challenge, 100% mortality with severe hepatic necrosis and hydropericardium was observed in the unvaccinated challenged group and in birds vaccinated with Company A. In contrast, birds vaccinated with Company B showed complete protection, with no mortality, no gross pathological lesions, and no mortality among contact birds. Commercial FAdV-4 vaccines differed markedly in protective efficacy. Elevated ELISA antibody titers alone were not predictive of protection, emphasizing the need for rigorous evaluation and careful selection of vaccines to effectively control HHS in broiler flocks.

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Introduction

Fowl adenoviruses (FAdVs), members of the genus Aviadenovirus within the family Adenoviridae, are widely distributed pathogens of poultry and have been associated with a variety of disease conditions, including inclusion body hepatitis (IBH), gizzard erosion, respiratory disease, and hepatitis–hydropericardium syndrome (HHS) (1,2). Among the recognized serotypes, fowl adenovirus serotype 4 (FAdV-4) is the principal etiological agent of HHS, a severe and often acute disease characterized by hydropericardium, hepatic necrosis, immunosuppression, and high mortality in broiler chickens (3-5).

Initially reported in South Asia, HHS has emerged as a major constraint to poultry production across Asia, the Middle East, and parts of Europe (4-6). Since approximately 2015, the re-emergence of highly virulent FAdV-4 strains—particularly novel or dominant genotypes—has caused widespread outbreaks in China, renewing interest in vaccine development (3,4,7,8). Molecular investigations indicate these virulent strains possess distinct genomic and antigenic features, including variations in fiber and hexon proteins, which likely influence pathogenicity and immune recognition (4,7,9).

Extensive experimental studies conducted in China have demonstrated that effective protection against virulent FAdV-4 challenge can be achieved, particularly with well-designed inactivated or recombinant vaccines (3,7,10). Inactivated oil-emulsion vaccines and subunit vaccines targeting Fiber-2 or its knob domain have been shown to induce robust protection, reduce virus shedding, and prevent clinical disease (7,10,11). However, vaccine efficacy has been reported to vary substantially depending on vaccine formulation, antigen selection, and challenge strain, highlighting the complexity of FAdV-4 immunoprophylaxis (1,7).

In Iran, FAdV-4 has been confirmed in broiler and breeder flocks, with molecular characterization revealing close genetic relationships with virulent Asian strains (12-14). Pathological investigations have documented classical HHS lesions, while serological surveys indicate widespread exposure at the flock level (11,13). Similar findings have been reported in neighboring regions, including Azerbaijan, further supporting the notion of regional dissemination of virulent FAdV-4 lineages across Eurasia (8).

Despite the availability of multiple commercial FAdV-4 vaccines, there is a notable lack of controlled comparative studies evaluating the efficacy of different commercial products under standardized experimental conditions, particularly using virulent challenge strains relevant to the Iranian and regional epidemiological contexts. Moreover, the relationship between pre-challenge ELISA antibody titers and actual protection against mortality and pathological lesions remains incompletely understood, especially when comparing vaccines from different manufacturers.

Therefore, the present study aimed to comparatively evaluate the immunogenicity and protective efficacy of two commercial FAdV-4 vaccines in broiler chickens following virulent challenge, using serological responses, mortality rates, pathological findings, and contact-bird outcomes as objective indicators of vaccine performance.

Materials and Methods

Experimental Design

One hundred one-day-old broiler chickens of the Arian strain were procured from a 32-week-old breeder flock with no history of vaccination against IBH or HHS. Birds were divided into four groups of 20, with five additional contact birds per group to monitor horizontal transmission. Group 1 served as unvaccinated and unchallenged as a negative control; Group 2 served as the unvaccinated and challenged as a positive control; Groups 3 and 4 were vaccinated with commercial vaccines A and B, respectively, at 21 days of age and challenged at 42 days of age (Table 1). Both commercial vaccines evaluated in this study were whole-virus inactivated FAdV-4 vaccines derived from virus propagated in embryonated chicken eggs and therefore contained the major structural antigens of FAdV-4.

Table 1. Vaccination and challenge protocols across the experimental groups.

Group	Treatment	Vaccination	Challenge
1	Control (No Challenge)	No	No
2	Control (Challenge)	No	Yes (Day 42)
3	FAdV-4 Vaccine (Company A)	Yes (Day 21)	Yes (Day 42)
4	FAdV-4 Vaccine (Company B)	Yes (Day 21)	Yes (Day 42)

Serology and Challenges

On day 42, blood samples were collected for ELISA detection of FAdV-4 antibodies using a commercial indirect ELISA kit (BioeStone Fowl Adenovirus Group I Antibody Test Kit, BioStone Animal Health Co., Ltd., China) according to the manufacturer's instructions. Subsequently,

at three weeks post-vaccination, birds in Groups 2, 3, and 4 were challenged intramuscularly with 0.5 mL of liver homogenate containing a field isolate of FAdV-4 from Iran ($10^{3.5}$ EID_{50}) according to the manufacturer's instructions. The challenge strain was a virulent Iranian FAdV-4 field isolate previously confirmed by molecular methods, which showed close phylogenetic similarity to currently circulating virulent Asian strains. However, complete genomic characterization and direct comparison with the proprietary vaccine strains could not be performed, as detailed sequence information was not disclosed by the vaccine manufacturers. Post-challenge, birds were monitored for seven days for clinical signs and mortality. Necropsies were performed on all deceased birds to identify gross lesions.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version XX (GraphPad Software, San Diego, CA, USA). ELISA antibody titers were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Results were expressed as mean ± SEM, and differences were considered statistically significant at $P < 0.05$.

Results

At 42 days of age, Group 4 (Company B) exhibited significantly higher mean ELISA titers (1440) compared with Group 3 (Company A) (910.5), Group 2 (576.1), and Group 1 (553.6) (Figure 1). Statistical analysis demonstrated a highly significant difference between Company B and all other groups ($p < 0.0001$). Vaccination with Company A also induced significantly higher titers than controls ($P < 0.05$). No significant difference was observed between the two control groups ($P > 0.05$) (Table 2).

Table 2. Comparison of ELISA antibody titers against FAdV-4 among the different experimental groups. Data are presented as mean ± SD; statistically significant differences between groups are indicated.

	Group 1	Group 2	Group 3	Group 4
Mean	553.6	576.1	910.5	1440
Std. Deviation	186.6	152.6	273.5	297.1
Std. error of Mean	62.19	50.85	86.48	93.96
Coefficient of Variation	33.70%	26.48%	30.03%	20.64%

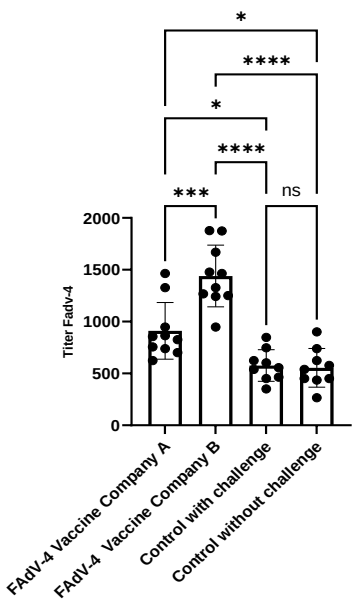

Figure 1. Comparison of ELISA antibody titers among the different experimental groups.

Following the challenges, Groups 2 and 3 experienced 100% mortality within 48 hours, displaying severe hepatic necrosis and hydropericardium. In contrast, Group 4 (Company B) showed no mortality or gross lesions within the first 48 hours, with only 20% cumulative mortality observed by day 7. Birds in the control Group 1 showed no mortality and gross pathological lesions at 48 hours or 7 days. Notably, 50% mortality occurred among contact birds in Group 3, indicating active horizontal transmission, whereas no mortality was observed among contact birds in Group 4 (Table 3). The survival proportions of the four groups are shown in Figure 2 (Figure 2).

Discussion

This study demonstrates a marked difference in protective efficacy between two commercial FAdV-4 vaccines despite identical administration and challenge protocols. While both vaccines induced humoral responses, only the Company B vaccine conferred substantial protection and prevented transmission to contact birds. In contrast, the Company A vaccine failed to prevent mortality, resulting in outcomes comparable to those observed in unvaccinated challenged controls.

These findings are consistent with international evidence indicating that successful protection against FAdV-4 is highly dependent on vaccine design and antigenic composition. In China, Pan et al. demonstrated that an inactivated vaccine prepared from a novel genotype FAdV-

4 strain provided effective protection against HHS challenge (3). Subsequent studies further established that vaccines targeting key structural proteins—particularly Fiber-2—can induce strong protective immunity and significantly reduce viral shedding (7, 10). Similar protective effects have been reported with oil-emulsion inactivated vaccines in Korea, which were capable of conferring cross-protection against multiple FAdV serotypes under experimental conditions (11).

Table 3. Mortality rates (%) in challenged and contact birds following virulent FAdV-4 challenge in vaccinated and control groups at 48 hours and 7 days post-challenge

		48 hours after challenge (Mortality rate)		7 days after challenge (Mortality rate)	
		Challenge	Contact Birds	Challenge	Contact Birds
1	Control + without challenge	0	0	0	0
2	Control + with challenge	100	0	-	-
3	FAdV-4 Vaccine Company A	100	0	-	50
4	FAdV-4 Vaccine Company B	0	0	20	0

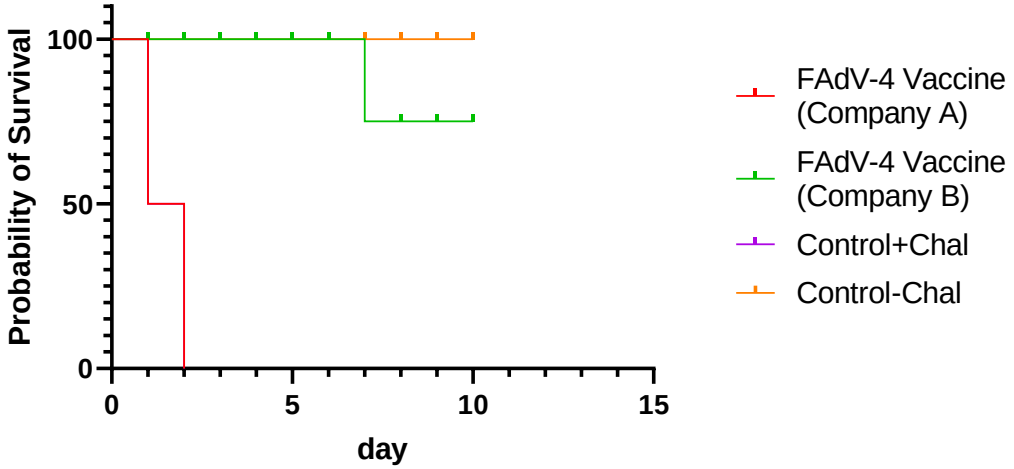

Figure 2. Survival proportions of birds in the different experimental groups.

However, not all vaccines provide equivalent protection. Liu et al. emphasized that differences in antigen selection, vaccine platforms, and adjuvant formulations can lead to substantial variation in immune outcomes (7). Although inactivated vaccines have been widely used to control FAdV diseases, technological advancements have led to the development of live attenuated and subunit vaccines as potentially more attractive and effective candidates (15). The present findings support this conclusion, as vaccination with the Company A product generated measurable ELISA titers yet failed to protect birds against lethal challenge.

A notable observation in this study is the discordance between ELISA antibody titers and survival outcomes. Although the Company A vaccine resulted in significantly higher antibody levels than those observed in unvaccinated controls, these antibodies were insufficient to prevent disease and death. This observation aligns with previous reports indicating that ELISA primarily measures binding antibodies and does not necessarily reflect the presence of neutralizing or protective antibodies (1, 7). Functional immunity against FAdV-4 likely requires antibodies directed against specific neutralizing epitopes and may also involve cell-mediated immune mechanisms, which are not captured by routine ELISA assays.

In contrast, vaccination with the Company B vaccine induced significantly higher antibody titers and provided complete protection, suggesting that both the magnitude and quality of the immune response are critical determinants of vaccine efficacy. Similar correlations between robust

humoral responses and protection have been documented in experimental Fiber-2-based vaccine studies (7, 10).

The failure of one commercial vaccine to protect against virulent challenge is particularly relevant in the Iranian context. Molecular studies from Iran have demonstrated the circulation of FAdV-4 strains closely related to highly virulent Asian lineages (12-14). These findings support the strain-matching concept, whereby vaccines derived from antigenically divergent strains may induce suboptimal protection against circulating field viruses (9). The complete protection observed with the Company B vaccine suggests that its antigenic composition may be better aligned with the challenge strain used in this study, although genomic comparison would be required to confirm this hypothesis.

The presence of mortality among contact birds in the Company A group further indicates active viral replication and shedding, which is consistent with reports that inadequate vaccination may fail to limit horizontal transmission (6, 7). In contrast, the absence of mortality among contact birds in the Company B group suggests effective suppression of viral spread, an essential feature for controlling HHS under field conditions.

Importantly, the similarity in baseline ELISA titers between Group 1 and Group 2 does not indicate pre-challenge infection or compromise the validity of the experimental model, because: Both groups remained clinically healthy prior to challenge, with no signs of HHS or adenoviral disease; post-challenge outcomes clearly differentiated the groups, with 100% mortality observed in Group 2 and no disease in Group 1, confirming a valid and functional challenge model; the ELISA assay used detects binding antibodies and may reflect low-level residual immunity without indicating active infection or protection. Therefore, baseline serological background in unvaccinated groups likely reflects maternal antibody status and/or field exposure and does not interfere with interpretation of vaccine efficacy or challenge outcomes.

Recent advances in FAdV-4 vaccine research have increasingly focused on recombinant and subunit vaccine platforms, particularly those targeting the Fiber-2 protein, which is considered a major protective antigen of FAdV-4. Recent studies have demonstrated that Fiber-2-based subunit vaccines can induce complete protection and significantly reduce viral replication and shedding following virulent challenge (16). In addition, newer vaccine strategies, including chimeric vector vaccines, dendritic cell–targeted fusion proteins, and multivalent recombinant constructs, have shown promising immunogenicity and broader protective potential under experimental conditions (7).

These developments support the concept that vaccine efficacy against FAdV-4 depends not only on the induction of detectable antibodies but also on antigen selection, structural conformation, induction of neutralizing immunity, and suppression of viral replication and shedding. The marked difference observed between the two commercial vaccines evaluated in the present study is therefore consistent with current understanding of the complexity of FAdV-4 immunoprophylaxis.

Collectively, these findings reinforce the need for evidence-based selection of commercial FAdV-4 vaccines, particularly in regions experiencing recurrent outbreaks. Reliance on serological responses alone may be misleading, and challenge-based efficacy data remain critical for vaccine evaluation. A limitation of the present study is the absence of quantitative viral load analysis. Future studies incorporating real-time PCR–based viral quantification and virus shedding analysis would provide a more comprehensive understanding of the relationship between vaccine-induced immunity, viral replication, and transmission dynamics.

Conclusion

In conclusion, the present study demonstrates that commercial FAdV-4 vaccines differ substantially in their ability to protect broiler chickens against virulent challenge. Only one of the tested vaccines provided effective protection and prevented horizontal transmission. These findings highlight the necessity of continuous surveillance and rigorous vaccine validation to effectively control HHS in poultry populations.

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Authors' Contributions

Arash Ghalyanchi Langeroudi: Investigation, formal analysis, writing – original draft preparation. **Hossein Hosseini:** Conceptualization, supervision, project administration, writing – review & editing. **Zahra Ziafati Kafi, Soroush Sarmadi, Fahimeh Jamiri, Alireza Bakhshi, Rima Morshed, Mahtab Heydarpour, Fatemeh Gharagazlou, Alireza Vafadar, Hirbod Sheikhi, Seyed Alireza Rezaee, Farnood Shahamati:** Methodology, validation, writing – review & editing. All authors read and approved the final manuscript.

Data Availability

The data presented in this study are available on request from the corresponding author.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. At the time the experiment was performed, formal ethical committee approval for poultry vaccine challenge studies was not mandatory at the authors' institution.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable.

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