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Comparative Histopathology and Immunogenicity of two Commercially Available Infectious Bronchitis GI-13 Vaccine Strains in Broiler Chickens

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ABSTRACT

Vaccine development is a lengthy process, and safety and efficacy studies are crucial before and after a vaccine's commercial use. The aim of this study was to evaluate the immunogenicity and histopathological effects of two commercially produced Infectious Bronchitis vaccines from the GI-13 lineage in young chickens. Seventy-five broiler chickens were divided into three groups of 25 (groups 1-3). At 21 days of age, the chickens were vaccinated through the ocular route. The first two groups were vaccinated against IB, while the last group served as the control group and remained unvaccinated. Using ELISA, the humoral immune response, as measured by antibodies, was assessed 3 weeks post-vaccination. Five days after vaccine administration, histopathology of the trachea and kidney was examined in vaccinated and control group chickens, and real-time PCR was performed to determine the RNA virus load in tracheal swabs. The results confirmed that both vaccines elicited serum antibody responses of 0.2618±0.1358 (Mean OD±Standard Deviation) in the first group and 0.2789±0.1164 in the second group, both of which were higher than those of the control group. Despite some variations between them, the difference was not statistically significant (p>0.05). However, the 793/B-like vaccine in group 2 had much higher histopathological lesion scores in the kidney (Group 1: 2.600±0.8944 and 3.800±0.4472 for Group 2) and the trachea (Group 1 scored a mean of 1.800±0.4472, while the second group scored a higher mean of 2.800±0.8367) (p<0.05), indicating that different vaccines may cause varying changes in the microscopic structure of tissues. Real-time RT-PCR analysis with 24 and 24.42 CT values for the first and second vaccinated groups, respectively, revealed no preference for vaccine strains, indicating similar viral loads. In conclusion, these findings suggest that both vaccines are immunogenic and, despite potentially having the same replication rate, one vaccine may be safer, causing less damage to target tissues in chickens. This means that choosing the right vaccine is important for controlling poultry diseases and managing poultry health.

Keywords: *Infectious Bronchitis Virus, GI-13, 793/B-like Vaccine, ELISA, Real-Time RT-PCR*

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1 Introduction

Infectious Bronchitis (IB) is caused by an enveloped single-stranded positive-sense RNA virus belonging to the genus Gamma Coronaviruses. The disease is a major concern in commercial poultry production worldwide (Nemr et al., 2025). The first site of viral replication is the upper respiratory tract. It replicates in epithelial cells lining the respiratory and urogenital systems, leading to clinical manifestations such as coughing, gasping, lacrimation, and sneezing in younger birds, and a decline in egg production in breeders and layers (Masoudi et al., 2020). Like most avian diseases, control relies on biosecurity and vaccination. Due to the high rate of viral mutation, several virus serotypes and genotypes are continuously reported worldwide.

Unfortunately, there is no 100% protection against heterologous viruses, and vaccinated birds may be infected by a different virus strain. Therefore, selecting the optimal vaccination protocol is critical for controlling infectious bronchitis (IB) (Ghetas et al., 2025). In general, vaccine strains are derived from local field viruses that have been carefully characterized, with all characteristic factors and genomes identified. To prepare a vaccine strain from field-isolated IB viruses, they are serially passaged in specific pathogen-free (SPF) embryonated chicken eggs to adapt the virus to the egg system, attenuate its pathogenicity, and maintain its immunogenicity (Nemr et al., 2025). Research shows that decreasing the virulence of the infectious bronchitis virus (IBV) through serial egg passage can result in inconsistent and unpredictable changes in its phenotype and genotype. Therefore, there is a need for more controlled methods to attenuate IBV to develop effective vaccines in the future (Benyeda et al., 2010; Kilany et al., 2025). IBV vaccine is a predisposing factor for several bacterial infections, especially colibacillosis caused by avian pathogenic E. coli (Callison et al., 2006). Field trials are necessary to assess the efficacy and safety of IBV vaccine strains in vivo.

As new strains of IBV continue to evolve globally, developing protective vaccines and protocols that establish a standard level of immunity and cross-protection against various strains has become a challenge for poultry researchers. Vaccination remains the most reliable way to prevent production losses (Awad et al., 2016). Vaccinated birds should not become sick, die from the vaccine itself, or suffer significant damage to their respiratory system or kidneys. There may be slight differences between two commercially developed vaccines derived from the same genotype or serotype of the Infectious Bronchitis virus (Abdel-Sabour et al., 2021). Therefore, some pathological and immunological responses may differ, such as coughing or respiratory problems following vaccination with one brand but not the other. Understanding these side effects and differences in immunogenicity can help veterinarians and farmers make informed decisions about which protocol and strain to use on the farm (Terregino et al., 2008). Multiple comparative IBV pathogenesis/vaccine studies already exist (including 793B/GI-13 and other serotypes) (Chousalkar et al., 2009; Ghetas et al., 2025; Kilany et al., 2025; Masoudi et al., 2020). However, this study is not a comprehensive efficacy or field-performance experiment, but a comparative evaluation of the early safety/immunogenicity of two commercial GI-13 vaccines, which was a unique and worthwhile investigation.

In Iran, the Infectious Bronchitis virus genotype GI-13, including 793B-like viruses, was first detected in 2007 and remained prevalent until the recent dominance of Variant 2 (Motamed et al., 2021). This strain has been controlled through several vaccinations using the same serotype virus vaccines, whether imported or locally developed. It is important to note that not all adverse effects post-vaccination are relevant to the vaccine virus itself, but also to factors such as management, flock health condition, age, and breed. The aim of this study was to investigate and evaluate any possible differences in humoral and pathological responses to two commercial IB GI-13 vaccines to understand the nature of the two strains from different companies but of the same virus genotype.

2 Material and Methods

2.1 Experimental design and animal housing

The trial was designed to compare the pathogenicity and humoral immunogenicity of Avishield IB GI-13 (Dechra) with a commercially purchased 793/B-like IBV vaccine already registered with the Iranian Veterinary Organization (IVO). Seventy-five one-day-old broiler chickens Ross 308 were purchased from a commercial hatchery and remained in controlled, conditioned, unvaccinated conditions until the beginning of the experiment at the age of 21. A Block randomization method was used to divide chickens into three groups of 25 (n=25 per group), and all were tested and confirmed to be free of infectious bronchitis virus (IBV) and other respiratory pathogens by molecular detection. All birds were of the same sex, and the mean body weight was 700±50 g. Chickens were housed in separate rooms under a

controlled environment (temperature: 24±2°C, humidity: 60±5%, 12-hour light/dark cycle). Food and water were provided ad libitum.

Group 1: Vaccinated with Avishield IB GI-13 (Dechra).

Group 2: Vaccinated with a locally produced 793/B-like IBV vaccine.

Group 3: Unvaccinated (Control)

2.2 Vaccination

Before conducting the experiment, the titers of both vaccines were evaluated and independently verified to ensure that all groups would be inoculated with the same virus titer. At 21 days of age, chickens were vaccinated via the ocular route with a single dose per bird, following the manufacturer's guidelines of 10^-5 EID50/mL (vaccines were administered at the same virus titer) (EID50/bird). Each vaccine was administered at the recommended dose of 50 µL per bird via the ocular route. Birds were monitored daily post-vaccination (pi) for signs of disease or any adverse reactions.

2.3 Sample Collection and Laboratory Analyses

2.3.1 Post-Vaccination Sampling (5 Days Post-Vaccination)

Five days after vaccination (26 days of age), 5 chickens per group were humanely euthanized by Cervical dislocation method. Tracheal and kidney tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin (H&E). Histopathological changes (e.g., inflammation, epithelial damage) were graded according to an established grading system (0=no lesions, 1=mild, 2=moderate, 3=severe) (Benyeda et al., 2010). All scoring and histopathological examinations were blindly conducted by a pathologist.

2.4 Serology by ELISA

At 42 days of age (21 days post-vaccination), the remaining chickens from all groups (n=20/group) were bled from the brachial vein. The blood was spun at 3000×g for 10 minutes, and sera were stored at −20°C until further investigation. An ELISA for IBV-specific antibody detection was performed using a commercial kit (Biocheck IBV Ab Test) according to the manufacturer's instructions. Sampled sera remained anonymous to avoid any possible bias. Optical density (OD) was measured, and antibody titers were quantified using the kit reference standards. Results were expressed as S/P±Standard Deviation (SD).

2.5 Real-time PCR

In addition, tracheal swabs were collected for real-time PCR analysis on the 5th day post-vaccination. Samples were assigned code numbers to remain anonymous until interpretation and were processed by the laboratory in a blinded manner. RNA was extracted using a commercial viral RNA isolation kit, Singapore RNA Extraction Kit (SinaClon, Iran). cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific) and random hexamer primers (SinaClon, Iran). Real-time PCR of the IBV 5' UTR gene was conducted. All real-time RT-PCR runs were carried out in an ABI 3600 instrument using the Realtime Master Mix Kit from Biotech rabbit. Initially, 5 µL of 4X Capitase Master Mix, 1 µL each of primer and probe, and 2 µL of cDNA were combined, and Nuclease-free water was added to a final volume of 20 µL. To validate the reaction process and primers, a positive control (793/B vaccine) and a negative control (Nuclease-free water as template) were included in each reaction set.

The optimal thermal cycle conditions were as follows: 95°C for 3 min, followed by 40 cycles at 95°C for 15 s and 60°C for 60 s (Table 1). Viral load was quantified using the cycle threshold (Ct) method; Ct values >40 were considered negative, and no viral detection was observed. Conversely, considering background and detection limits, any sample with a Ct value of 15 or higher was considered positive. Therefore, a lower Ct value indicates higher viral levels than a higher Ct value.

Table 1. Oligonucleotide sequence of primer set (Callison et al., 2006)

IBV5´GU391	GCT TTTGAGCCT AGC GTT	391–408(nt)
IBV5´GL533	GCCATGTTGTCACTG TCTATTG	533–512(nt)
IBV5´G probe	FAMCACCACCAGAACCTGTCACCTCBHQ1	494–473(nt)

2.6 *Statistical Analysis*

All statistical analyses were conducted using GraphPad Prism (v9.5) and Excel software. Given a small number of samples, non-parametric tests were used for Histopathology and PCR. Antibody titers and viral load: One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare between-group differences in IBV-specific antibody titers and viral load. Results are reported as Mean±Standard Error (SE) of the mean. Histopathology scores: Nonparametric data were compared using the Kruskal-Wallis test, followed by Dunn's post hoc test. Statistical significance was determined with a p-value of ≤0.05 for all tests.

3 Results

3.1 *Histopathology*

Kidney Lesions

Kidney tissue sections were evaluated for histopathological lesions after immunization. The average histopathological score of the Avishield IB GI-13 (Dechra) group was 2.600±0.8944, while the 793/B-like vaccine group had a significantly higher score of 3.800±0.4472. The unvaccinated control group had the lowest histopathological score (1.600±0.5477; p<0.05). Statistical analysis showed that Avishield IB GI-13 (Dechra) was significantly different from the 793/B-like vaccine (p<0.05), with the latter inducing more severe kidney lesions. Additionally, the difference between the 793/B-like vaccine and the control group was highly significant (p<0.001), while no significant difference was observed between Avishield IB GI-13 (Dechra) and the control group. These results suggest that although both vaccines trigger similar serological responses, the 793/B-like vaccine leads to more severe kidney histopathological changes than Avishield IB GI-13 (Dechra), indicating a potentially better safety profile for the latter (See Figures 1 and 2).

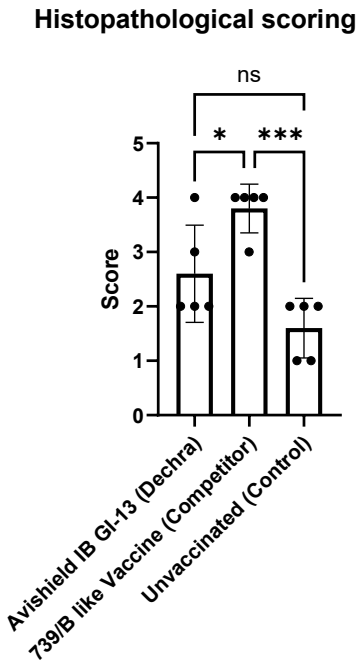
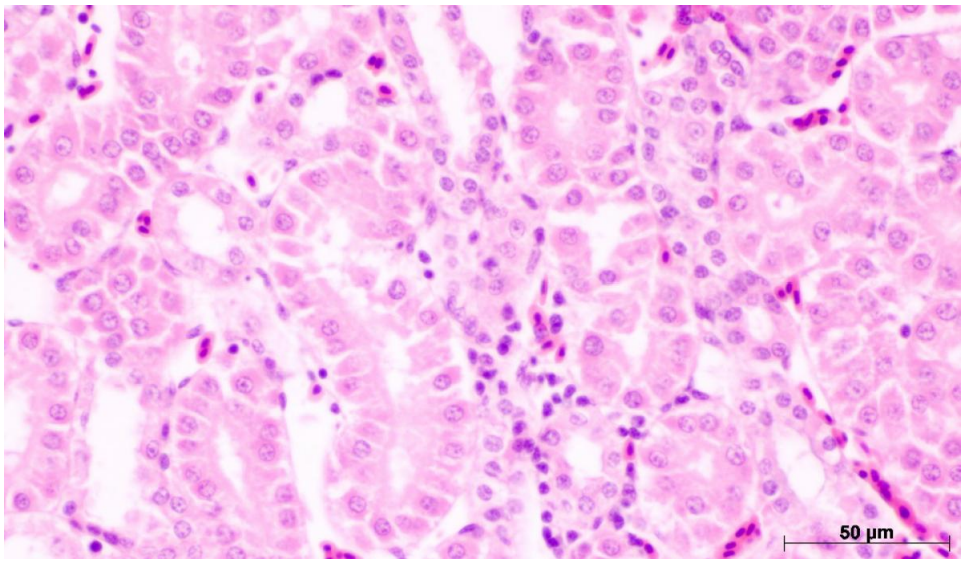


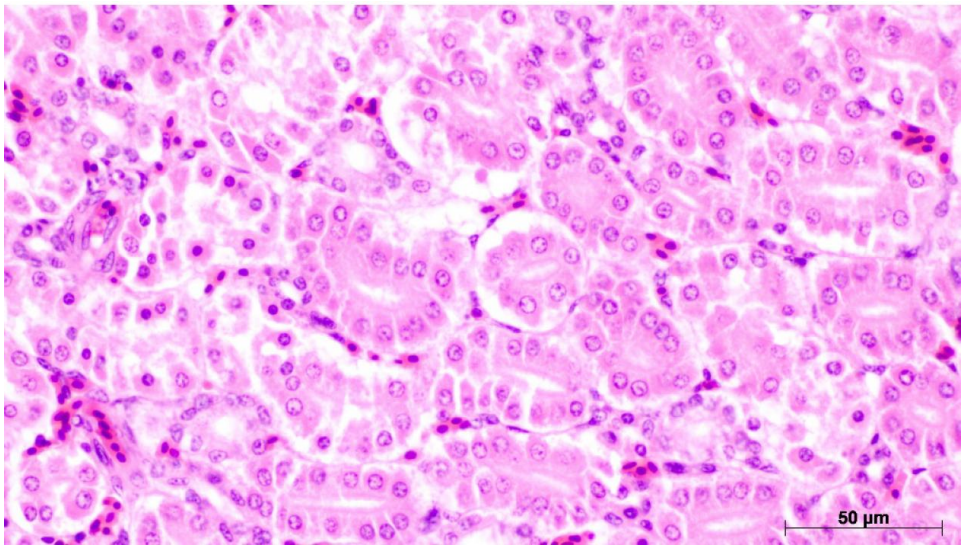
Figure 1. Histopathological evaluation of both Vaccines virus effects on the kidney in the two groups in comparison with the control. The scatter dot plot, including Mean±SD error bars, shows histopathological evaluation of both vaccine virus effects on the kidney in the two groups compared with the control. Histopathology scores were significantly different between the two treatment groups (p<0.05) and between

the two treatment groups and the control (p<0.001). Asterisks indicate statistically significant results. *<0.05, ***<0.001.A nonparametric test was used due to the small sample size.

Unvaccinated (Control)



Avishield IB GI-13 (Dechra



739/B like Vaccine

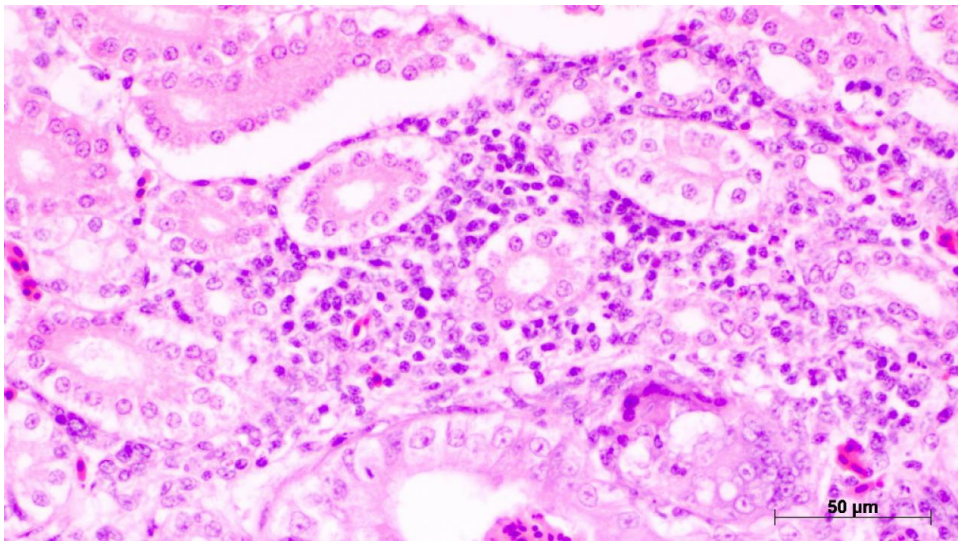


Figure 2. Kidney tissues lesions in both the experiment and control group 5 days after vaccination (H&E staining). Intact kidney tubular and nephron epithelia in the control group are seen. In experiment groups, various degree of loss of epithelial cells and degeneration, local and diffuse lymphocyte infiltration, especially in the 793/B group, is seen.

3.2 Tracheal lesions

Histopathological scoring of the trachea revealed that the Avishield IB GI-13 (Dechra) group scored a mean of 1.800±0.4472, while the 793/B-like vaccine group scored a higher mean of 2.800±0.8367. Statistical analysis showed no significant difference (ns) between the Avishield IB GI-13 (Dechra) and the 793/B-like vaccine(p>0.05), but the 793/B-like vaccine induced significantly more severe tracheal lesions compared to the unvaccinated control group (p<0.01). The trachea in the unvaccinated group was intact. These data suggest that although both vaccines elicit similar serological responses, the 793/B-like vaccine (group 2) has a greater pathological impact on the kidney and trachea than Avishield IB GI-13 (Dechra). Lower histopathological scores in the Avishield IB GI-13 (Dechra) group indicate greater safety regarding pulmonary and renal tissue destruction (Figures 3 and 4).

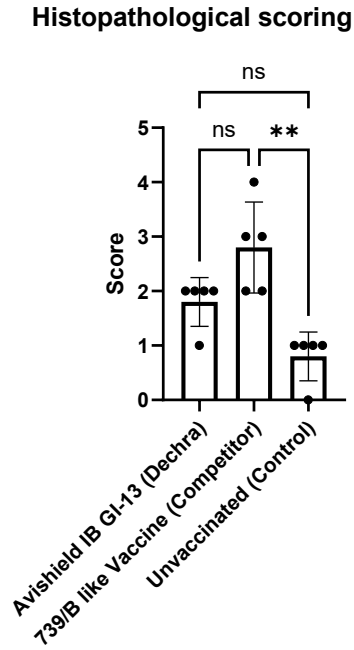


Figure 3. Histopathological evaluation of both vaccine virus effects on the trachea in the two groups in comparison with the control. The scatter dot plot, including *Mean±SD* error bars, shows histopathological evaluation of both vaccine virus effects on the Trachea in the two groups compared with the control. Tracheal histopathology scores between the two treatment groups were not significant (p<0.05), but the two treatment groups (p<0.05) in comparison with the control were significant (p<0.01).

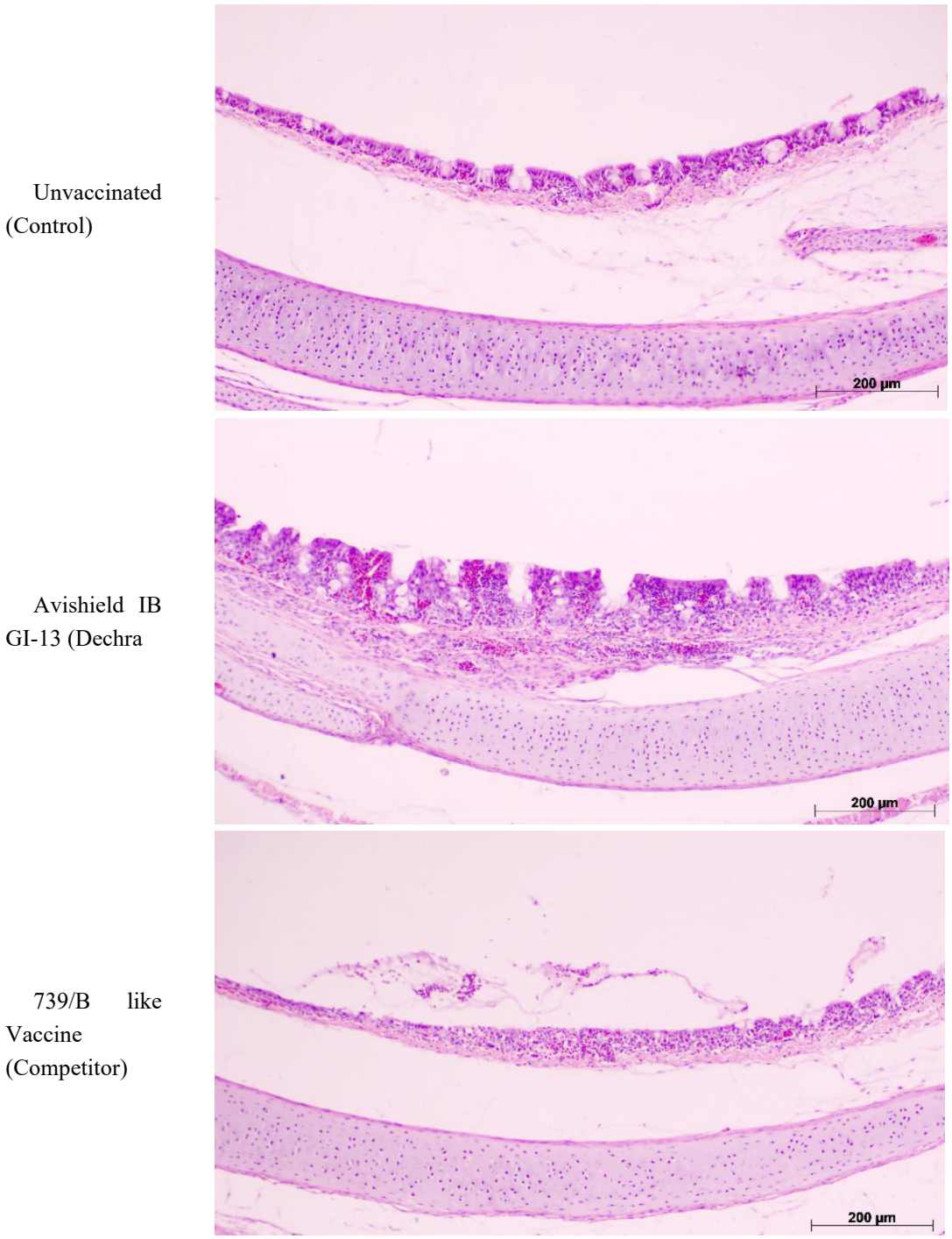


Figure 4. Tracheal tissue lesions in both the experiment and control groups, 5 days after vaccination (H&E staining). Intact tracheal epithelium is evident in the control group. In experimental groups, varying degrees of epithelial cell loss and degeneration, especially in the 793/B group, were observed, with lymphocyte infiltration and edema beneath the mucosa**.**

3.3 Serology

The humoral immune response specific to IBV in chickens was evaluated by measuring the S/P ratio using the ELISA Biocheck assay after vaccination with Avishield IB GI-13 (Dechra) and the 793/B-like vaccine. The group vaccinated with Avishield IB GI-13 (Dechra) had a mean S/P ratio of 0.2618±0.1358 (SD), while the group vaccinated with the 793/B-like vaccine had a higher mean S/P ratio of 0.2789±0.1164 (SD). The unvaccinated control group had a significantly lower mean S/P ratio of 0.1302±0.01106 (SD).

Statistical analysis did not show any significant differences (ns) between the Avishield IB GI-13 (Dechra) and the 793/B-like vaccine groups, indicating similar levels of seroconversion with both vaccines. However, both vaccine groups produced higher antibody titers compared to the unvaccinated control group. These findings suggest that Avishield IB GI-13 (Dechra) and the 793/B-like vaccine induce comparable serological responses in chickens when administered via the ocular route at 21 days of age, without prior infection or vaccine boosting (Figure 5).

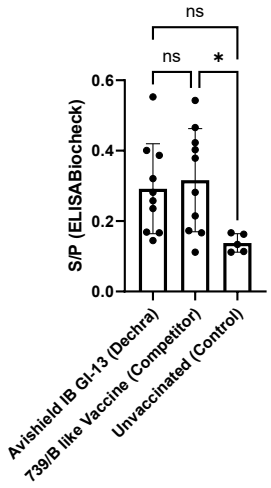


Figure 5. Antibody response to vaccination against infectious bronchitis, evaluated by ELISA, was higher in both experimental groups than in the control group. Vertical bars represent standard deviations, and significant differences between treatment groups and the control are marked with *($p \leq 0.05$).

3.4 Real-Time RT-PCR:

Although both vaccinated groups (Avishield IB GI-13 and 739/B-like) have considerably lower CT values (approximately 24) compared to the unvaccinated control (approximately 43, already negative), the lower CT values in both vaccinated groups also suggest the presence of viral RNA, indicating that some degree of viral replication is taking place. The average CT values for Avishield IB GI-13 (24.00) and the 739/B-like vaccine (24.20) are extremely close to each other, indicating similar viral RNA levels and hence, similar viral loads in these groups. The key point is that reduced CT values indicate greater viral RNA and, hence, increased viral load, so both vaccines achieved similar viral loads in the trachea at 5 days post-vaccination, indicating comparable levels of replication in this tissue at this time point (Figure 6).

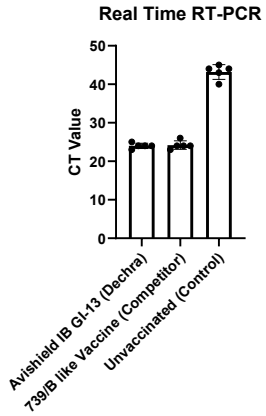


Figure 6. Real-time PCR results for tracheal swabs 5 days post-inoculation, based on Ct values. Both vaccine groups have the same Ct value, but the higher Ct value in the control group indicates a negative result.

4 Discussion and Conclusion

This study compares early histopathology lesions and serology responses in vaccinated, unchallenged broilers. Several studies have reported the efficacy and pathological effects of various IB vaccines (14). Awad et al. in 2016 studied the efficacy of an IB vaccination regimen composed of H120+793/B against VAR2 challenge and reported 80% protection in the ciliostasis test (Awad et al., 2016). A recent study reported that vaccination with H120 + D274 could enhance immunogenicity compared with vaccination with H120 alone (Abdel-Sabour et al., 2021). A prime-boost vaccination program using IB-Ma5 and IB-793B provided satisfactory protection against IBV QX strain in SPF and commercial broiler chickens; however, about 50% of the commercial broilers were shedding the challenge QX strain with relatively high titers (Terregino et al., 2008)

Sultan et al. (2019) examined 6 vaccination regimes in broilers with M41, Variant2, and Ma5, and found the highest tracheal lesion scores in groups primed or primed and boosted with the 793B live attenuated vaccine. The same effects were observed for kidney scores: primed or boosted vaccines with 793 B and Ma5 induced more renal histopathological lesions. They described various lesions, including Hyperplasia and degeneration of the trachea epithelium and nephrosis, along with hemorrhage in the kidneys, 7 days after challenge with the field virus (Sultan et al., 2019). Barari et al.'s study compared histopathological lesions caused by IB88, H120, and IB4-91 vaccines in broiler chickens aged 1 to 5 days. Hyperemia and infiltration of inflammatory cells in the kidney and lung, hyperemia and infiltration of inflammatory cells in the lung, necrosis of respiratory epithelium, and hyperplasia of tracheal mucous cells were observed in the experimental groups. Differences in inflammatory cell infiltration, tracheal mucous cell hyperplasia, and renal hyperemia were significant between IB4-91 and H120 (Barari et al., 2024).

Vaccines are not always safe or without side effects, especially in farm conditions. Depending on the virus's serotype, it can have many pathological effects on vital organs, leading to reduced egg production and quality. The virus also causes renal damage and is considered a predisposing factor for respiratory bacterial infections, especially airsacculitis (Eid et al., 2024). Although it has been widely accepted that attenuated IBV vaccines induce varying degrees of ciliostasis depending on the level of attenuation (Awad et al., 2016; Jackwood et al., 2015; Lee et al., 2004), the effect of IBV attenuated vaccines on the trachea must be considered when measuring their protective efficacy. ELISA is a common tool for evaluating antibodies in poultry sera after vaccination against Infectious Bronchitis or when a field infection is suspected. However, it is not clear if the antibody response detected by ELISA accurately reflects the level of protection provided by different IBV vaccination programs against IBV challenges. Nonetheless, it is a common measure for assessing vaccine-induced immune responses and an indicator of whether a vaccine can stimulate the immune system (Sjaak De Wit et al., 2011). Various studies have used ELISA for efficacy or protection assessments (Abdel-Sabour et al., 2021; Jackwood et al., 2015; Masoudi et al., 2020). The current study evaluated the antibody response and acute histopathological lesions of two genetically related IBV vaccines belonging to the genotype (GI-13) but produced by two different companies in two different territories. The focus was on their performance in broiler chickens in controlled conditions. The study found that all vaccinated groups developed significantly higher IBV-specific antibody titers than the control group. However, the antibody response is not the gold standard for vaccine efficacy. It does explain how much the immune system was stimulated by the vaccine, leading to a more protective effect.

The 4/91 virus or 793B serotype was first reported in Britain in 1991. Since then, there have been many reports of this serotype from various parts of the world. Detection of 793 B-like viruses in Iran dates back to 2007, and until 2017, it was the most prevalent IBV reported from poultry flocks in Iran, accounting for more than 67% of detections (Motamed et al., 2021).

One of the most widespread IB serotypes globally and a common vaccine type against IB disease is the 793B-like viruses. Recent research suggests that this vaccine can protect chickens not only against homologous strains but also heterologous strain challenges when combined with another serotype, such as Mass type viruses simultaneously (Sjaak De Wit et al., 2011).

Ocular administration of the IB vaccine stimulates the Harderian gland, located behind the eye socket, resulting in a robust immune response (Jeurissen et al., 2000).

Based on the data from another study, the 793/B.08IR vaccine effectively protects Specific Pathogen Free (SPF) chickens from infection when exposed to a virulent strain of the Infectious Bronchitis Virus (IBV) called 793/B, under the specific conditions of the experiment (Masoudi et al., 2020).

Although several experiments have been done on various IB vaccines in Iran, the present study is research on the early safety/immunogenicity of two commercial vaccines that offer a thorough comparative assessment of Avishield IB GI-13 (Dechra) and a 793/B-like IBV vaccine in broiler chickens, focusing on their immunogenicity and acute histopathological effects, which have not been investigated before in Iran. Both vaccines elicited similar humoral immune responses, as evidenced by comparable antibody titers detected through ELISA, demonstrating their capacity to induce successful seroconversion. However, histopathological examinations showed that the 793/B-like vaccine resulted in significantly more severe kidney and tracheal lesions than Avishield IB GI-13, suggesting potentially greater acute histopathological effects from the former (Almayahi et al., 2022). The presence of viral RNA in both vaccinated groups was demonstrated by real-time RT-PCR, indicating limited viral replication after vaccination and no excessive virus persistence. These findings underscore the significant advantage of Avishield IB GI-13 over the 793/B-like vaccine, demonstrating greater immunogenicity and reduced tissue pathology, making it an improved vaccine for IBV vaccination programs in chickens. The focus of this study was on the early response to a live attenuated IB vaccine; however, the lack of a field virus challenge was a missing procedure, so the protection level and duration couldn't be assessed, and the limited time points resulted in a small sample size. Another limitation of this study is that although ELISA is a reliable test for evaluating antibody response after vaccination or as a surveillance protocol, and has even been used in several experiments, it cannot serve as a correlative criterion for bird protection or efficacy. For further research into vaccine safety, it is suggested to assess lesion resolution at a later time point (e.g., 10-14 days) to strengthen the case for safety, especially if one vaccine shows quicker recovery.

While the results of this study are promising, further studies are warranted to examine the long-term protection of immunity, field efficacy under diverse environmental conditions, and prolonged use in commercial poultry flocks. More research is needed to determine their side effects when combined with other serotypes, as observed in field conditions.

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Use of Artificial Intelligence

During the preparation of this manuscript, the authors used AI-assisted tools (ChatGPT-4) for language editing and refinement of specific sections to improve clarity and readability. Following the use of these tools, the authors thoroughly reviewed and revised the content and take full responsibility for the final version of the manuscript.

Conflict of Interest

We declare that no conflict of interest.

Author Contributions

A.G.L. and H.H. designed and directed the project; Z.Z.K., H.H., and A.G.L. conceived and planned the experiments. A.B., S.S., F.J., and N.S. carried out the experiments. A.B., S.S., and H.H. planned and carried out the simulations. A.B., S.S., F.J., Z.Z.K.And N.S. contributed to sample preparation. H.H., N.M., and A.G.L analyzed the data. A.G.L., Z.Z.K., and H.H. contributed to the interpretation of the results. N.M. took the lead in writing the manuscript with input from all authors. All authors discussed the results and commented on the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

We hereby declare that all ethical standards have been respected in the preparation of the submitted article, and the study adhered to ARRIVE guidelines for in vivo research reporting. This study was approved by the Research Ethics Committee of the Razi Vaccine and Serum Research Institute, Karaj, Iran (Code: IR.RVSRI.REC.1402.005).

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