Iraqi Journal of Veterinary Sciences

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Immune and immune-histochemical response for bivalent attenuated vaccines against Newcastle and infectious bronchitis diseases in broilers

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Article information

Article history:
Received 05 February, 2025
Accepted 12 March, 2025
Published 16 March, 2025

Keywords:
Attenuated vaccines
Newcastle disease
Infectious bronchitis
Broilers

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Abstract

This study intended to research the immunological and immune-histochemical outcomes of 6 bivalent live vaccines against NDV and IBV. Two hundred ten, 1-day old chicks divided equally into 7 groups. The chicks of group 1 received (the Nobilis MSD® vaccine). G2 vaccinated with (Hipraviar® vaccine). G3 performed with (Vaxxinova® vaccine). G4 given (Komipharm® vaccine). G5 vaccinated with (KAVAC® vaccine). G6 vaccinated by (Dagluban® vaccine). Group 7 (negative control). The groups G1 to G6 were vaccinated by the ocular route at 1 and 14 days of age with the abovementioned vaccines. Serum samples were collected for ELISA and HI tests, while tracheal tissue was collected for histochemical and immunohistochemical Staining. The high titer of Abs against NDV was observed mainly in G1 on days 21, 28, and 35 when compared to other groups of different ages. IBV ELISA findings showed that G1 had a greater Abs at day 21 than G4 and G6, whereas G2 explained a significantly higher titer of Abs at day 28 than other groups. The HI results indicate a bimodal and fluctuating titer of Abs. Assessment of tracheal mucin pictures found that MUC5AC and MUC5B immunostaining were significant in group 5 after 7 and 21 days. MUC5AC immunostaining was high in more than half of the epithelial cells in group 2. In conclusion, some of the tested vaccines provided a sufficient amount of Abs against NDV and IBV diseases. Mucin illustrated high to moderate expression in the epithelial surface and goblet cells associated with groups of experiments.

DOI: 10.33899/ijvs.2025.157260.4111,

Introduction

The poultry industry is a crucial sector of the economy that significantly contributes to the food supply in many developing nations, including Iraq, where industrial chicken production began in 1905 (1). Diseases are considered one of the most significant production problems in the poultry industry, as poultry farms suffer from continuous bacterial, viral, and fungal infections (2). Newcastle disease (ND) and Infectious bronchitis (IB) are significant viral illnesses in chickens with considerable economic repercussions worldwide (3,4). The Newcastle disease virus (NDV), belonging to the genus Avulavirus in the family Paramyxoviridae, induces Newcastle disease (ND) (5). In contrast, the IB virus induces infectious bronchitis, a genus of the Gammacoronavirus genus, Coronaviridae family, and Orthocoronavirinae subfamily. The same family and subfamily classify IBV, also known as avian coronavirus, as severe acute respiratory syndrome (6,7). Both viruses possess genomes composed of single-stranded RNA (ssRNA). In contrast, the RNA of NDV is negative-sense, and IBV's is positive-sense (8). The single-stranded composition of their genome enables these two antigens to develop rapidly, resulting in significant genetic heterogeneity across circulating viral strains (9). This is particularly evident in IB, where recombination plays a role in genetic diversity (10). IB and ND impact poultry of all ages and breeds; however, the severity of the diseases

fluctuates with the age of the birds, with IB being more severe in young progeny (3,6). Meanwhile, the severity of ND is more evident in chickens of all ages (11). Live attenuated vaccines are the most common vaccination to protect against these viruses. These vaccines have the potential to transmit live viruses. It is also hard to control IBV through vaccination because the spike (S) protein always changes. This creates many different antigens and serotypes that don't protect against each other. This is a significant limitation (12). It is possible that various isolates may not give full cross-defense towards each other, even though all NDV strains belong to the same serotype. This is attributed to the significant genetic diversity (13,14). These mutations necessitate developing vaccines that effectively combat these two viruses. Other important challenges during immunization against ND include handling and storage, particularly in tropical regions (15).

The mucosa of the larynx, trachea, and bronchi express two main respiratory mucins, MUC5AC and MUC5B; these two types have been identified only in the dorsal tracheal region of the cranial and middle trachea of chickens, providing as a physical barrier to microbial attachment and consequent infection-related events. Hence, this research project planned to evaluate the antibody's response to the commonly found bivalent live attenuated NDV-IBV vaccines and identify mucin expression in tracheal tissue after immunization.

Materials and methods

Ethical approval

The Committee of Scientific Morals issued the endorsement certificate with the number UM.VET.2024.036 on September /2024 granted the College of Veterinary Medicine the moral authority to perform this planned work.

Experimental design

Two hundred ten, seven groups of one-day-old broilers (Ross 308) were allocated randomly, each consisting of 30 chicks, and kept in separate enclosures with supplies and a bed of wood shaving material. The chicks of group 1 received (ND-IB vaccine -Nobilis MSD®). G2 were vaccinated with (ND-IB vaccine-Hipraviar®). G3 were performed with (ND-IB vaccine-Vaxxinova®). G4 were given (ND-IB vaccine-Komipharm®). G5 was vaccinated with (ND-IB vaccine-KAVAC®). The G6 group was immunized with (ND-IB vaccine-Dagluban®). Meanwhile, in group 7, chicks were maintained as a control group and were not vaccinated. As mentioned, the groups from G1 to G6 were vaccinated by ocular route at 1 and 14 days of age with different commercial bivalent vaccines.

Serum samples

Two ml of blood from the wing vein of chicks was collected at 1, 7, 14, 21, 28, and 35 days of age to obtain serum (4 chicks from each group and age) (16). The blood samples were centrifuged at 1500 rpm for 15 minutes and stored in appropriately labeled vials at -20°C for serological tests (17).

Indirect ELISA

Per the manufacturer's recommended protocol, sera were tested to estimate the level of Abs against NDV and IBV using an indirect ELISA test kit for both illnesses as an indicator parameter. of systemic immunity (18-20).

Hemagglutination Inhibition test

In a 96-well micro-titer plate, 50 µL of each serum sample had been diluted with 50 µL of PBS (2-fold dilution). Each well was subsequently injected with 50 µL of (NDV) or (IBV-treated by trypsin) (21) antigen (4HAU) (GD Academy/Netherlands), thoroughly mixed, and kept to sit at room temperature for 30 minutes. The micro-titer plate was then filled with 50 µL of 2% chicken RBC solution, which was carefully mixed. To detect the antibody titer, the micro-titer plate was eventually incubated for 45 minutes at room temperature (22).

Immunohistochemical staining

Samples from birds (trachea) of experimental groups were extracted at 5, 7, 19, and 21 days of age. Tissue sections from this organ were obtained and preserved in 10% neutral buffered formalin. In paraffin wax, tissue samples were placed and sectioned at 4 µm (23). The sections were selected for immunohistochemical analyses. The sections were deparaffinized with a series of xylene and rehydrated in a different descending ethanol series. The MUC5AC and MUC5B primary antibodies used in the immunohistochemical staining were considered for paraffin-embedded samples. MUC5AC (MUC5AC Polyclonal Antibody, Elabscience, USA) and MUC5B (MUC5B Polyclonal Antibody, Elabscience, USA) were stained with Poly-HRP detection system (Elabscience, USA) (24). Before adding the primary antibodies, the slides were boiled for 10 min in an oven. After that, the slides were incubated overnight in the cold room with the primary antibody, and the secondary Poly-HRP anti-rabbit antibodies (Elabscience, USA) were used. Then, the slides were developed with DAB, after which the slides were counterstained with hematoxylin. The digital images were collected and qualified using a digital camera and the ImageJ software. The degree of immunostaining was calculated as the percentage of mucin expression by examining MUC5AC and MUC5B proteins in the trachea.

Histochemical Staining

Histochemical staining was achieved using PAS staining to identify and pinpoint mucus in the trachea. The glycogen was depleted, and the sections were deparaffinized and incubated with the enzyme. After that, sections were washed

and ready for the PAS staining (25). The digital images were collected and qualified by using a digital camera.

Growth performance

The experiment's chicks were fed a basic diet prepared per the broiler's standard requirements (26). Weekly measurements of the initial and final weights of the body, total feed intake per bird, and food conversion ratio (FCR) were made to identify any differences between the experiment's groups (27-29).

Statistical analysis

To analyze the data (SPSS version 22.0) was used. The estimated Abs titer values against NDV, Abs titer against IBV, FCR, and weight gain parameters were calculated using Duncan's test ($P \leq 0.05$) and shown as mean values±standard error (SE) (30).

Results

The level of Abs titer against NDV and IBV in experimental groups was determined using the ELISA technique. Antibodies titer against NDV in the groups of the experiment (Table 1) shown compared to G3, G4, and G6 demonstrated that G5 produced a much higher titer of Abs at 7 days of age. The high titer of Abs was observed in G1 on days 21, 28, and 35 (2097.7±843.1, 5591.2±2485.3, 4180.5±1659.6) when compared to other groups at different ages ($P \leq 0.05$).

Antibodies titer against IBV in the groups of the experiment; statistically, no significant differences in Abs titer were given by all groups at day 7, but compared to G1, G4, and G5, G2 showed a significantly high titer of Abs at day 14. In addition, G1 had a significantly higher titer of Abs at day 21 compared to G4 and G6, whereas G2 demonstrated a higher titer of Abs at day 28 compared to other groups. On days 14, 28, and 35, G2 had a higher titer of Abs than other groups of varying ages (584.7±237.2, 3159.7±308.5, and 2189.7±462.2) ($P \leq 0.05$) (Table 2). Table 3 illustrates HI data and a bimodal and varied titer of Abs. As a result, the HI titer significantly elevated in G5 at 7 days (5±0.57 log2) and in G1 at 21 and 35 days (4.66±0.33, 6±0.57 log2).

According to the HI assay for finding IBV Abs, table 4 demonstrates that G2 has a high titer of Abs at 7 and 35 days (5.33±0.33,5.66±0.33). In contrast, at 21 days, G1 has the highest titer (7±0.57) compared to other groups. The food conversion ratio values in table 5 varied by group age. At 14 days, G1 (1.08±0.01) showed the greatest value, followed by G2 (1.19±0.007), G4 (1.13±0.007), and G7 (1.15±0.01). In contrast to the other groups, G7 (1.25±0.01) (1.89±0.01) and G2 (1.40±0.01) (1.48±0.1) had the greatest values at 28 and 35 days, respectively, while G2 (1.43±0.01), G6 (1.43±0.007), and G7 (1.45±0.01) showed the lowest value ($P \leq 0.05$) at 21 days.

Table 6 results revealed variations in the experimental groups' weekly weight gain. At seven days of life, there is a significant difference ($P \leq 0.05$) between the G7 (197.2±3.9) and G1 (177.1±5.6). The difference between the G6 and G1 at 14 days of age was (501.7±11.7) against (460±12.9). There aren't any notable differences between the groups at age 21 days. A remarkable discrepancy exists between the G5 and G2 at 28 days of age (1689.7±60.1) and (1519.2±60.4). There are no appreciable differences between the groups at age 35 days.

Table 1: Antibodies titer against NDV in groups of experiment (Mean±SE) by ELISA

Groups	1 day	7 days	14 days	21 days	28 days	35 days
G1	18243.7±4837^{a}	3515.7±766.4^{ab}	1102.5±436.6^{ab}	2097.7±843.1^{a}	5591.2±2485.3^{a}	4180.5±1659.6^{a}
G2	18243.7±4837^{a}	4844.2±732.6^{ab}	1930.5±353.3^{a}	1511.5±544.7^{ab}	861.7±325.9^{b}	1881.7±483.7a^{b}
G3	18243.7±4837^{a}	3163.7±834.6^{b}	1238.5±319.6^{ab}	506.5±38.5^{b}	1123.2±346^{b}	958.7±403.2^{b}
G4	18243.7±4837^{a}	2437±894.6^{b}	1222±149^{ab}	415.5±61^{b}	403.2±136.2^{b}	1231.7±813.1^{ab}
G5	18243.7±4837^{a}	5907.2±385.2^{a}	1001.5±256.5^{b}	1341.2±452.1^{ab}	1899±1586.6^{b}	1734.2±1182.3^{ab}
G6	18243.7±4837^{a}	2779.7±1109^{b}	755±199.7^{b}	420.7±61.3^{b}	230±110.5^{b}	304.6±75.7^{b}
G7	18243.7±4837^{a}	2358.7±925.3^{b}	590.2±113.9^{b}	271.5±24.1^{b}	36± 20.3^{b}	33.3±22.2^{b}

A significant difference at $P<0.05$ is observed by values with separated letter superscripts in the same row.

Table 2: Antibodies titer against IBV in groups of experiment (Mean±SE) by ELISA

Groups	1 day	7 days	14 days	21 days	28 days	35 days
G1	6885.7±683^{a}	1081.7±357.7^{a}	93±54^{b}	4705.5±2547^{a}	2345±617.1^{ab}	2368.7±600.1^{a}
G2	6885.7±683^{a}	1653±405.1^{a}	584.7±237.2^{a}	1413±413.2^{ab}	3159.7±308.5^{a}	2189.7±462.2^{a}
G3	6885.7±683^{a}	1288±425.8^{a}	261.5±111.8^{ab}	3523.7±993.9^{ab}	1650.7±523.8^{ab}	1804±305.1^{a}
G4	6885.7±683^{a}	1129.7±378.6^{a}	131.2±56.4^{b}	878.5±309.6^{b}	1858±337.1^{ab}	3119.5±973.1^{a}
G5	6885.7±683^{a}	1084±461.5^{a}	145.7±24.2^{b}	3369.2±921.2^{ab}	2981.5±839.3^{ab}	2028±250.2^{a}
G6	6885.7±683^{a}	815.2±3331.7^{a}	250.5±168.8^{ab}	982.7±282.1^{b}	1895.7±366.6^{ab}	1677.3±206.5^{a}
G7	6885.7±683^{a}	1525.7±529.6^{a}	118.2±91.9^{b}	129.2±21.4 ^{c}	144.5±23.8^{b}	133.6±20.8^{b}

A significant difference at $P<0.05$ is observed by values with separated letter superscripts in the same row.

Table 3: Antibodies titer (log2) against NDV in groups of the experiment (Mean±SE) by HI test

Groups	7 days	21 days	35 days
G1	5±0.57^{bc}	4.66±0.33^{a}	6±0.57^{a}
G2	6.33±0.57^{ab}	3.66±0.33^{a}	4±0.57^{b}
G3	4.33±0.57^{c}	1.33±0.66^{bc}	1.66±0.88^{cd}
G4	4±0.0^{c}	1.33±0.88^{bc}	3±0.0^{bc}
G5	6.66±1.52^{a}	3±0.57^{ab}	4±0.57^{b}
G6	4.33±0.57^{c}	1.3±0.33^{bc}	1±0.57^{d}
G7	4±0.57^{c}	0.33±0.33^{c}	0.33±0.33^{d}

A significant difference at P<0.05 is observed by values with separated letter superscripts in the same row.

Table 4: Antibodies titer (log2) against IBV in groups of the experiment (Mean±SE) by HI test

Groups	7 days	21 days	35 days
G1	4±0.0^{bc}	7±0.57^{a}	5.33±0.33^{ab}
G2	5.33±0.33^{a}	5±0.57^{b}	5.66±0.33^{a}
G3	5±0.0^{ab}	6.33±0.33^{ab}	3.66±0.33^{cd}
G4	5±0.57^{ab}	1.66±0.66^{c}	4.66±0.33^{abc}
G5	3.66±0.33^{c}	6±0.57^{ab}	4.33±0.33^{bcd}
G6	1.33±0.33^{d}	1.3±0.88^{c}	3.33±0.33^{d}
G7	0.66±0.33^{d}	0.33±0.33^{c}	0.66±0.66^{e}

A significant difference at P<0.05 is observed by values with separated letter superscripts in the same row.

Table 5: Food conversion ratio (mean±SE) for experiment groups at 7, 14, 21, 28, and 35-day

Groups	7 days	14 days	21 days	28 days	35 days
G1	1.05±0.007^{a}	1.08±0.01^{a}	1.35±0.01^{a}	1.42±0.01^{d}	1.59±0.01^{c}
G2	1.05±0.01^{a}	1.19±0.007^{c}	1.43±0.01^{bc}	1.40±0.01^{cd}	1.48±0.1^{a}
G3	1.05±0.01^{a}	1.1±0.01^{a}	1.41±0 .01^{b}	1.38±0.007^{c}	1.52±0.1^{b}
G4	1.05±0.01^{a}	1.13±0.007^{b}	1.37±0.01^{a}	1.31±0.01^{b}	1.59±0.01^{c}
G5	1.05±0.01^{a}	1.08±0.005^{a}	1.37±0.01^{a}	1.38±0.01^{c}	1.59±0.01^{c}
G6	1.06±0.01^{a}	1.08±0.01^{a}	1.43±0.007^{bc}	1.29±0.01^{b}	1.82±0.007^{d}
G7	1.05±0.01^{a}	1.15±0.01^{b}	1.45±0.01^{bc}	1.25±0.01^{a}	1.89±0.01^{e}

A significant difference at P<0.05 is observed by values with separated letter superscripts in the same row. The smallest value of FCR equals the significant value.

Table 6: Weight gain (mean±SE) for experiment groups at 7, 14, 21, 28, and 35-day

Groups	7 days	14 days	21 days	28 days	35 days
G1	177.1±5.6^{b}	460±12.9^{b}	954.1±29.3^{a}	1579.3±51.8^{ab}	2185.2±76^{a}
G2	189.1±4.3^{ab}	466.2±13^{ab}	910.7±27.3^{a}	1519.2±60.4^{b}	2162.7±95.1^{a}
G3	183.1±3.5^{ab}	466.1±9.8^{ab}	921±15.1^{a}	1552.4±22.9^{ab}	2184.4±47^{a}
G4	190±5.7^{ab}	464.1±12.7^{ab}	948.1±16.7^{a}	1661.5±33.2^{ab}	2300±41.4^{a}
G5	185.1±3.9^{ab}	482±12.5^{ab}	988.2±32.1^{a}	1689.7±60.1^{a}	2337.4±82.8^{a}
G6	191.4±5.8^{ab}	501.7±11.7^{a}	982±22.4^{a}	1656.9±53.1^{ab}	2184.5±81.8^{a}
G7	197.2±3.9^{a}	475.5±13.5^{ab}	908.2±43.8^{a}	1650±52.9^{ab}	2151.7±90.1^{a}

A significant difference at P<0.05 is observed by values with separated letter superscripts in the same row.

Using immunohistochemical techniques, we estimated the degree of mucin in trachea sections after chicks received different bivalent attenuated vaccines against Newcastle and infectious bronchitis diseases. Positive and negative expression of MUC5AC and MUC5B in the tissue is valuable to qualify the degree of mucin expression. In all sections, Mucins were noticed in the epithelial surface and superficial epithelial goblet cells at the distal part of the trachea (Figure 1A, B). The immunostaining of the MUC5AC was high expression in groups 2 and 5, weak expression in groups 1, 3, 4, and 6, and negative expression in group 7 after 7 and 21 days from vaccination (Figure 1A). The immunostaining of the MUC5B showed high expression in groups 2, 4, and 5, weak expression in groups 1, 3, and 6, and negative expression in group 7 after 7 days from vaccination. However, after 21 days, MUC5B had a high expression in group 5 only, weak expression in all other groups, and negative expression in group 7 (Figure 1B). Analysis of the tracheal mucin images demonstrated that the immunostaining of MUC5AC and MUC5B were dominant in group 5 after 7 and 21 days (Figure 1C, D). Immunostaining of MUC5AC was positively staining in more than 50% of the epithelial cells in group 2 (Figure 1C). However, the MUC5B protein was stained in about 60% of the cells after the first dose of vaccine in groups 2 and 4 (Figure 1D). In addition, all the tracheal surface epithelium was analyzed to determine the mucin expression using PAS staining. The mucin had a high expression in the epithelial surface and superficial epithelial goblet cells in groups 2 and 5, moderate expression in groups 1, 3, and 4, weak expression in group 6, and negative expression in group 7 after 7 and 21 days from vaccination (Figure 1E).

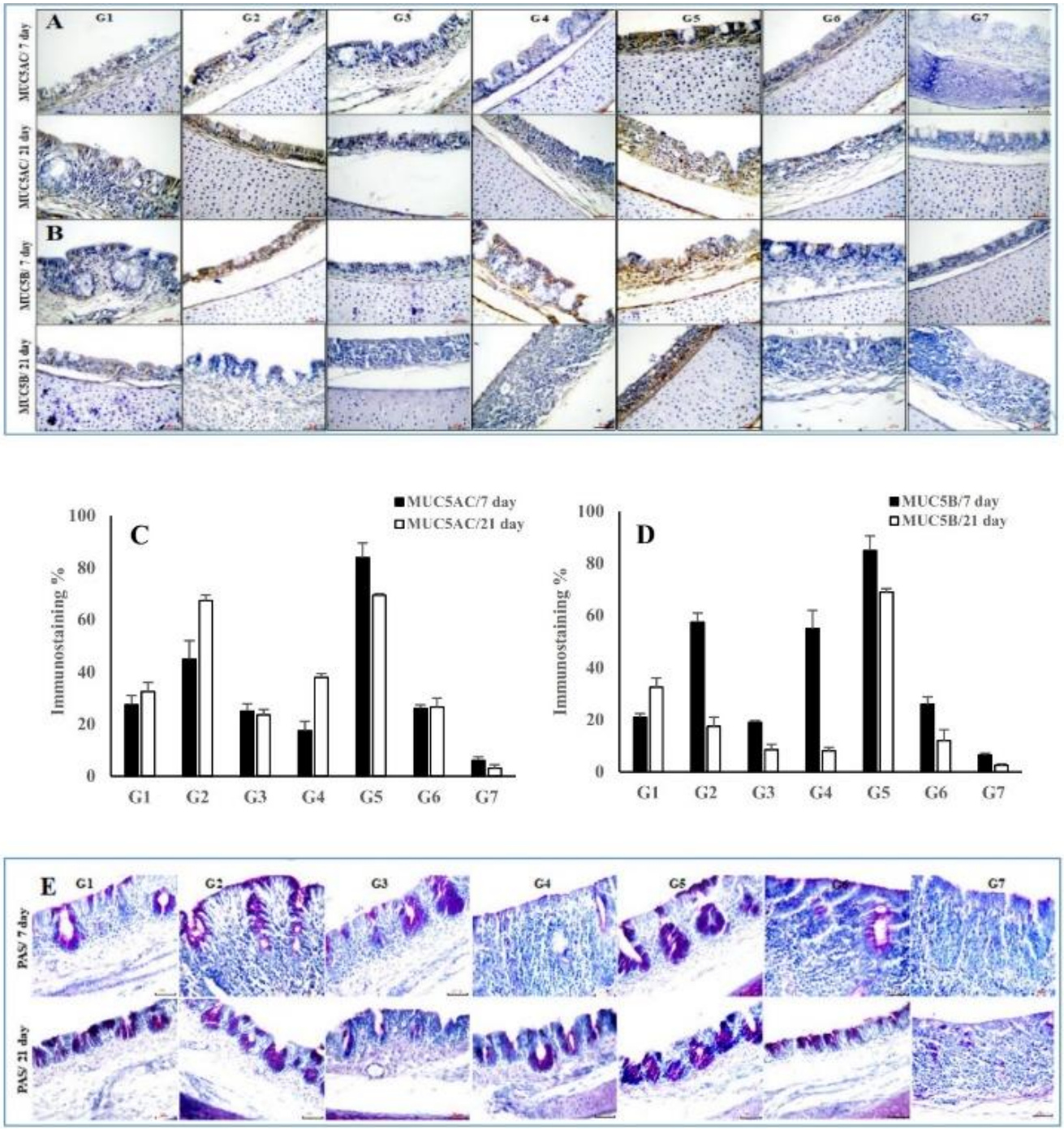

Figure 1: Detection of mucin by examining MUC5AC and MUC5B proteins in the trachea. A. Expression of MUC5AC in the tracheal mucosa, which detects the secreted mucin as a dark stain mainly located at the epithelial cells of the trachea after 7 and 21 days from vaccination in different groups (20µm IHC). B. Expression of MUC5B in the tracheal mucosa, which detects the secreted mucin as a dark stain mainly located at the epithelial cells of the trachea after 7 and 21 days from vaccination in different groups (20µm IHC). C. Quantification of the percentage of MUC5AC immunostaining in the positive stain epithelial cells of the trachea after 7 and 21 days in different groups by using ImageJ software. D. Quantifying the percentage of MUC5B immunostaining in the trachea-positive stain epithelial cells after 7 and 21 days in different groups using ImageJ software. E PAS stain of mucins in the epithelial cells of trachea, secreted mucins were stained as red after 7 and 21 days from vaccination in different groups (20µm PAS).

Discussion

Poultry, one of the most widely reared domesticated animal types, contributes more than 30% of animal protein production (31). The huge economic losses caused by disease outbreaks demonstrate the economic importance of the chicken business (32). Live attenuated and deadly vaccines are two of the most significant approaches for reducing disease outbreaks. The most extensively used vaccines worldwide are live vaccines, which are manufactured from viral strains classified by serotype. Live attenuated vaccines elicit strong protective antibody responses, making them appropriate for use in regions where the Newcastle disease and infectious bronchitis are prevalent (6,33).

The ELISA test findings at one day of age (table 1) revealed no significant variation in ND Abs levels between groups, which matches the results of Al-Jobori *et al.* (34). These findings support the conclusion that passive immunity is often transient, lasting 1-2 weeks and frequently less than 4 weeks. The ELISA test findings at 14 days of age revealed no significant differences between the groups; however, the second, fifth, and sixth groups were favored by a non-significant difference. One explanation for this could be the received maternal Abs, which could influence the vaccine's efficacy during the first two weeks of life. This finding agrees with the conclusions of Kabiraj *et al.* (35), who said that some strains used in the immunization campaign and how the vaccine is administered might affect the responses and that maternal immunity can supplement vaccination. The ELISA test findings showed a substantial decline in the third, fourth, and sixth groups at 21 days of age (36). Data from the ND Abs titer showed that G1 vaccinated chicks had greater mean titers at 28 and 35 days of age than other vaccinated groups (37). The high titer of Abs in G5 at 7 days may presented due to no interference with maternal Abs and rapid stimulation of immune response.

ELISA results in table 2 are commonly used in IBV serological investigations based on their efficiency, sensitivity, rapidity, and competence for large-scale applications (38). Chicks demonstrated a high level of anti-IBV MDA at the onset of the study. On day 21 of the experiment, contrary to monovalent vaccination with either the Ma5 or 4/91 strain, it was apparent that combining both vaccine strains provided higher cross-protection against heterologous IB viruses when applied to chickens (39). Titer of Abs to IBV declined with all groups (40) when they claimed that vaccinated birds showed a significant rise in the antibody level at 2 weeks post-vaccination. The findings of Awad *et al.* (41), who mentioned that the Abs titer was elevated at 7 days and diminished at 14 days post-vaccination with different strains of IBV, are in line with our findings regarding IBV vaccines, which demonstrate that the Abs titer increases with all vaccines of the experiment at 7 days post-vaccination and decreases dramatically at 14 days. Furthermore, a previous study reported that vaccination with a live Mass-type vaccine (classical strain) at one day of age and then administered a 793B (variant strain) vaccine two weeks later offered good protection against IBV (42).

The findings of Table 3 are in line with a study conducted by Smialek *et al.* (43); each SPF and commercial chick displayed significantly higher HI antibody titers compared with the correct control groups. Chhabra *et al.* (44) concluded that MDA does not affect IgA production in the upper respiratory tract after vaccination with the H120 strain alone or combined with CR88 in day-old commercial broiler chickens; these results align with our research data. According to the results of the HI test (43), the group vaccinated with the classical strain of IBV showed no evidence of any statistically significant variations in mean HI antibody compared to the control group. In contrast, the group immunized with the variant strain displayed a notable increase in mean HI Abs titer when contrasted to other groups; thus, the varied titer of Abs between groups may depend on the types and concentration or titer of antigens in each vaccine.

The outcomes of table 4 are further supported by research indicating that intraocular delivery of all vaccines results in more protection than drinking water vaccinations (45). In line with Bordoloi *et al.* (46), our outcomes match their conclusion that the mean HI titer of all vaccinated groups was greater than 5 log2 and was high until the end of the experiment, sufficient for protecting the birds from significant clinical manifestations. Mean HI antibody titers over 3log2 have been demonstrated to protect against NDV Bordoloi *et al.* (46). The lack of product efficiency may explain the low concentrations of Abs seen with some of the vaccines mentioned above (47).

From our observations in Table 5, as stated by Rauf *et al.* (48), broilers who had received vaccinations more frequently experienced decreased feed efficiency, adversely affecting their performance. The stress of the vaccination in this experiment may have caused the release of corticosterone into the bloodstream, contrary to the findings of the FCR data. Wegner *et al.* (49) indicated that the type of protective program may affect the final body weight of chicks, which is consistent with the weight gain results in Table 6. In their study, another author stated that multiple vaccination schedules against IB, IBD, and ND showed differences in FCR in Cobb500 chickens after the 42-day experiment. The unvaccinated broiler group had the highest body weight and a superior FCR compared to the other broiler groups that obtained immunization throughout the study (50). Another study found that vaccination of healthy chickens with different live and/or inactivated NDV vaccines had little or no effect on the production parameters (51).

The mucus in the airway is important for covering the respiratory tract. The respiratory barriers coated with mucus protect against microorganisms (52). Increasing expression of mucin genes is caused by metaplasia or hyperplasia of the

cells that express mucus, for example, goblet cells and glandular mucous cells. It is generally known that different mucin genes are expressed in various respiratory diseases (53). The airway layer is rich in the two important mucin genes, MUC5AC and MUC5B (54,55). In this study, the immunostaining and PAS staining show a very low mucin expression in group 7, whereas strong mucin expression in groups 2 and 5 and moderately expressed in other groups. These results confirm that the degree of mucin recognition is a fundamental tool for identifying a good vaccine that leads to an increase in the trachea's mucus. In addition, our results showed that mucin expression plays an important part in goblet cell metaplasia and airway inflammation after vaccination in some groups. This is the first paper designating the vital role of MUC5AC and MUC5B expression to compare the degree of mucin expression in response to different types of vaccines in poultry.

Conclusions

The low titer from several evaluated vaccines for the ND-IB antigen expressed that the bivalent vaccines used in the present investigation exhibited differences in antibody titer between groups. Immunohistochemical protocols detected both positive and negative expressions for MUC5AC and MUC5B in the tissue with different levels of mucin expression.

Acknowledgments

The authors thank the University of Mosul's College of Veterinary Medicine for supporting this study.

Conflict of interest

The researcher declares no conflicts of interest related to publishing the current research.

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الاستجابة المناعية والمناعية النسجية للقاحات المضعفة الثنائية ضد مرض النيوكاسل والتهاب الشعب الهوائية المعدي في الدجاج اللاحم

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الخلاصة

صممت الدراسة الحالية لتقييم النتائج المناعية والمناعة النسجية لستة من اللقاحات الحية الثنائية ضد فيروس مرض النيوكاسل والتهاب القصبات الخمجي. تم تقسيم الأفراخ وعددها مئتان وعشرة إلى سبعة مجاميع. تم تحصين المجموعة الأولى بلقاح نوبيليس. تم تحصين المجموعة الثانية بلقاح هيبرافير. حصنت المجموعة الثالثة بلقاح فاكسينوفا. تم تحصين المجموعة الرابعة بلقاح كوميفارم. حصنت المجموعة الخامسة بلقاح كافاك. وأخيرا حصنت المجموعة السادسة بلقاح دكلوبان. أما المجموعة السابعة فكانت مجموعة السيطرة السالبة. تم تحصين المجاميع من الاولى إلى السادسة بواسطة التقطير بالعين بعمر واحد وأربعة عشر يوما باللقاحات المذكورة أعلاه. جمعت عينات المصل لاختبار الاليزا وتثبيط التلازن، بينما تم جمع أنسجة القصبة الهوائية لفحص المناعة النسجية. لوحظ ارتفاع مستوى الأضداد لمرض النيوكاسل بشكل رئيسي في المجموعة الأولى في الأيام واحد وعشرون وثمانية وعشرون وخمسة وثلاثون يوما عند مقارنتها بالمجاميع الأخرى. أظهرت نتائج اختبار الاليزا لفيروس التهاب القصبات الخمجي أن المجموعة الأولى كان لديها مستوى أعلى من الأضداد في اليوم الواحد والعشرون مقارنة بالمجموعتين الرابعة والسادسة. تشير نتائج تثبيط التلازن إلى مستوى متباين من الأضداد في المجاميع. بينت المقاطع النسجية للكشف عن المخاط في القصبة الهوائية بواسطة مؤشري المخاط MUC5ACوMUC5B بان النتائج في المجموعة الخامسة بعمر سبعة وواحد وعشرون يوما كانت معنوية وملحوظة. كانت فعالية التصبيغ بواسطة المؤشر MUC5AC مرتفعة في أكثر من نصف الخلايا الظهارية للمجموعة الثانية. إن محصلة التجربة البحثية بينت كفاءة بعض اللقاحات وذلك بقدرتها على إنتاج كمية أكبر من الأجسام المضادة ضد مرض النيوكاسل والتهاب القصبات الخمجي، وأظهرت مادة المخاط تعبير متوسط الى مرتفع في النسيج الظهاري والخلايا الكأسية في مجاميع التجربة.