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Comparative evaluation of early humoral and cytokine responses induced by four commercial infectious bursal disease vaccines in broiler chickens

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Abstract

Objective

Infectious bursal disease virus (IBDV) is an important viral pathogen of broiler chickens because of its tropism for immature B lymphocytes in the bursa of Fabricius and its potential to cause immunosuppression. The current study compared the initial immune responses created by 4 commercially available IBD vaccines in broiler chickens through stimulation of both serological and cytokine responses.

Materials and methods

A total of 250 one-day-old Ross 308 broiler chicks were randomly divided into 5 groups of 50 birds and allocated into their respective treatment received one of four types of commercial vaccine (G1-G4) and the control (G5) (Didn't receive vaccine). The antibody response of the immune system was assessed using indirect ELISA on day 1, 10, 15, 20 and 25. Quantitative RT-PCR (qRT-PCR) was performed on days 1-25 to measure the relative expression of IL-1 beta and IFN-gamma. In addition, the field isolate used (used in challenge study) was identified as containing IBDV by conventional RT-PCR targeting *VP2*.

Results

The maternal antibody titers for each group are similar at 1 day old. At 10 and 15 days of age, Group 1 had the highest antibody titer with the initial humoral response at those times and approached the highest final titer at 25 days. Both Groups 2 and 3 had an intermediate antibody titer at 20 days of age greater than that of Group 1. The expression levels of *IL-1β* did not vary significantly between groups but Group 1 had the greatest fold increase as measured by the number of individuals who were part of the analysis. Conversely, the level of *IFN-γ* expression was significantly different between groups. To summarize, the commercial IBD vaccine candidates examined differed in the timing and the intensity of their resultant immune responses.

Of the 3 groups, Group 1 had the most favorable early immunogenicity profile by inducing earlier humoral antibody development, a stronger expression of *IFN-γ*, and the highest final IgY titer.

Conclusion

The findings suggest the use of integrated immune kinetics could be used as a means to generate comparative immunogenicity data of IBD vaccines in broiler chicken populations.

Keywords: Gumboro disease, *IL-1β*, *IFN-γ*, vaccine immunogenicity, *VP2* gene

Paper Type: Research Paper.

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Introduction

Infectious bursal disease (IBD), commonly known as Gumboro disease, remains an important viral disease of chickens because of the costs associated with the disease as well as its effect on suppression of the immune system in broilers that are being raised. Infectious bursal disease is caused by infectious bursal disease virus (IBDV), which targets immature B lymphocytes located in the bursa of Fabricius (the major source of immature B lymphocytes) and therefore inhibits the normal development of humoral immunity, leading to increased risk of succumbing to secondary infections (Dey et al., 2019; Alkie & Rautenschlein, 2016). While the primary tool for IBD management is vaccination, various factors are also important when evaluating a vaccine's immunogenicity, such as vaccine strain, the age of the bird, the presence of the circulating field virus, and maternally derived antibodies. In young chicks, maternal antibodies can help temporarily protect them from disease, but they may hinder vaccine response and delay the development of an active immune response; thus, early evaluation of the vaccine in broiler production systems is critical (Naqi et al., 1983; Solano et al., 1986). This is why it is important for studies evaluating the immunogenicity of vaccines to measure both the amount of an antibody early on after vaccination and the time that it takes for antibodies to show up. In other words, when comparing different vaccines for IBD, some commercial vaccines will be much different than one another in terms of their early immunogenicity and biological performance when used in the same conditions (Hamad et al., 2020; Alkie & Rautenschlein, 2016). In addition to measuring an antibody response (humoral response), measuring cytokine markers (e.g., *IL-1β* and *IFN-γ*) can give very useful information related to vaccine-induced immunologic activation,

which may explain the variability in the responses among birds (Qin & Zheng, 2017; Liu et al., 2010). At the molecular level, the *VP2* gene has been one of the most informative areas to explore when studying IBD viruses (IBDV) due to both its diagnostic and antigenic relevance to these viruses. Moreover, livestock production in general and chicken production in particular plays a vital socio-economic role for people living in low-income countries of Africa and Asia. Chickens are widely distributed avian species around the world, due to their short generation interval and adaptability in a wide range of agro ecologies. The chickens provide high-quality protein and income for the poor rural households and are the most widely kept livestock species in the world. This is due to the presence of the valuable traits of chicken like disease resistance, adaptation to harsh environments and ability to utilize poor quality feeds. We hypothesized that commercial IBD vaccines would differ in the kinetics and magnitude of vaccine-induced humoral and cytokine responses in broiler chickens. Therefore, the aims of this project were to compare the timing and magnitude of humoral and cytokine responses induced by four commercial IBD vaccines (measured through ELISA, cytokine gene expression studies, and conventional RT-PCR using the *VP2* gene) in broiler chickens.

Materials and methods

Field isolate collection and confirmation: The veterinary hospital in Babylon Province, Iraq was used to obtain an isolate from the field of infectious bursal disease virus (IBDV) from a number of broiler flocks that were showing clinical and pathological signs of IBD. The birds were showing signs of depression and ruffled feathers, as well as a watery, whitish diarrhea. The major gross lesions that were seen at necropsy included an enlarged and edematous bursa of Fabricius with haemorrhagic lesions, as well as haemorrhages in the muscles of the thigh. Aseptic procedures were used to obtain samples from infected birds. Blood samples were drawn for serological testing and tissue samples were taken from the bursa of Fabricius to confirm molecularly. Blood samples were collected for serological testing, whereas bursal tissue samples were collected for molecular detection of IBDV. Viral RNA was extracted from the bursal tissue samples, and IBDV was detected by RT-PCR targeting the *VP2* gene. Representative positive amplicons were subsequently selected for sequencing.

Experimental birds and housing: This study was conducted utilizing a total of 250 one-day-old Ross 308 broiler chicks that have been raised under typical husbandry practices in an experimental house with controlled conditions according to group housed separately. Feed and water were provided on an ad libitum basis during the study period. Environmental conditions including temperature, ventilation, and lighting were maintained according to standard broiler production management techniques.

Experimental design and vaccination protocol: The chicks stood randomly assigned into five equal groups (50 birds per group). Four groups were vaccinated on the first day of age with different commercial IBD vaccines, whereas the fifth group was maintained as an unvaccinated control. The vaccination protocol was as follows: Group 1 (G1): Vaksimue IBD MVN 002 strain, 0.1 mL/chick, subcutaneous injection, single dose at one day of age, Group 2 (G2): Vaxxon IBD, 0.25 mL/chick, subcutaneous injection, single dose at one day of age, Group 3 (G3): BursaPlex

vaccine, 0.25 mL/chick, subcutaneous injection, single dose at one day of age, Group 4 (G4): CAVAC Transmune vaccine, 0.25 mL/chick, subcutaneous injection, single dose at one day of age, and Group 5 (G5): Unvaccinated control group. All groups received the conventional routine flock vaccines used under local production conditions, including Newcastle disease, infectious bronchitis, and avian influenza vaccines, in addition to the experimental IBD vaccination schedule.

Sampling schedule: Blood samples were collected from representative birds in each group at 1, 10, 15, 20 and 25 days of age. Samples collected at these time points were used for serological evaluation and cytokine gene-expression analysis. For ELISA, serum samples from 10 birds per group at each time point were analyzed. For gene-expression analysis, whole-blood samples from 5 birds per group at each time point were used for RNA extraction and downstream qRT-PCR. Bursa of Fabricius samples were collected from representative challenged birds for molecular confirmation and pathological examination.

Serological assay by indirect ELISA: The humoral immune response against IBDV was assessed using a commercial indirect ELISA kit for the detection of anti-IBDV antibodies in chicken serum. Microtiter plates pre-coated with inactivated IBD antigen were used according to the manufacturer's instructions. Serum samples were diluted 1:500 by adding 1 µL of serum to 0.5 mL of sample diluent and mixed thoroughly. Then, 100 µL of diluted serum samples, positive controls, and negative controls were added to the designated wells. The plates were incubated at room temperature (22-27°C) for 30 min, washed four times with wash buffer, and incubated with enzyme-conjugated anti-chicken IgG (IgY) for a further 30 min. After washing, 100 µL of substrate reagent (pNPP chromogen) was added and incubated for 15 min at room temperature. The reaction was stopped by adding 100 µL of stop solution, and absorbance was calculated at 405 nm by means of an ELISA reader. Antibody titers were calculated according to the kit manufacturer's instructions.

Preparation of challenge virus: The challenge virus was prepared from a local IBD outbreak isolate collected in Al-Hilla City, Babylon Province, from broiler chicks showing typical clinical disease and mortality. The inoculum was prepared from infected bursae of Fabricius. Briefly, 5 g of bursal tissue was homogenized with 10 g of sterile sand in a mortar, followed by addition of 45 mL phosphate-buffered saline (PBS; pH 7.4). The homogenate was centrifuged at 2500 rpm for 20 min, and the supernatant was collected and recentrifuged under the same conditions. Penicillin (1000 IU/mL) and streptomycin (10 mg/mL) were added to the final supernatant to reduce bacterial contamination, and the inoculum was stored at −20°C until use.

Challenge test: At 16 days of age, five birds from each experimental group were randomly selected and orally challenged with 0.2 mL/bird of the prepared viral suspension. The challenged birds were monitored for five days post-challenge for the appearance of clinical signs, morbidity, and postmortem changes. This challenge model was used as a comparative post-challenge assessment of the early protective immunogenicity of the tested vaccines against a locally confirmed field isolate of IBDV.

Viral RNA extraction and conventional RT-PCR for *VP2* detection: Conventional RT-PCR was performed at the Biotechnology Research Center/BIO-GEN Laboratory, Al-Qadisiyah,

Iraq, for confirmatory detection of IBDV and amplification of the *VP2* gene region. Viral RNA was extracted from representative bursal samples using a commercial viral RNA extraction kit according to the manufacturer's instructions. The extracted RNA was either used immediately or stored at −80°C until analysis. Reverse transcription was carried out using AMV reverse transcriptase after thermal denaturation of RNA at 94°C for 5 min followed by immediate cooling on ice. Amplification of the *VP2* region was performed using the primer pair Vvfp 775 (forward) and Vvrp 1028 (reverse). PCR amplification was conducted in a final reaction volume of 50 µL under the following cycling conditions: initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 45 s, and extension at 60 °C for 1 min; followed by a final extension at 60°C for 10 min. PCR products were separated by electrophoresis on 1% agarose gel prepared in Tris-acetate-EDTA buffer, stained with ethidium bromide, and visualized under ultraviolet illumination. Representative positive amplicons were selected for sequencing analysis.

qRT-PCR **for cytokine gene expression:** To evaluate the immunological response induced by the tested vaccines, qRT-PCR was used to determine the relative expression of interleukin-1 beta (IL-1β) and interferon-gamma (*IFN-γ*), using β-actin as the housekeeping reference gene. Total RNA was extracted from whole-blood samples collected from 5 birds per group at each sampling time using a commercial total RNA extraction kit. RNA concentration and purity were assessed spectrophotometrically before cDNA synthesis. Complementary DNA was synthesized using a commercial reverse-transcription kit according to the manufacturer's recommendations. qRT-PCR reactions were prepared in a final volume of 20 µL containing BlasTaq™ 2× qRT-PCR Master Mix, 0.5 µL of each forward and reverse primer, template cDNA, and nuclease-free water. The thermal-cycling conditions consisted of enzyme activation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Primers specific for chicken IL-1β, *IFN-γ*, and β-actin were designed using NCBI reference sequences. The 2^−ΔΔCt comparative method was used to measure relative gene expression. In this method, β-actin was used as a reference gene. The unvaccinated control group at the time of sampling was also considered as a calibrator. In the analysis of relative quantification, the PCR amplification efficiency was included. Each sample was analyzed in technical replicates and the average Cq value was used for subsequent calculations.

Statistical analysis: Data were analyzed using SAS statistical software. Results were expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) was used to evaluate the effects of experimental group and sampling age. Duncan's multiple range test was used for post hoc comparisons among group means. Differences were considered statistically significant at $P < 0.05$.

Results

Humoral immune response assessed by ELISA: The ELISA results demonstrated marked differences in antibody titers among the experimental groups over time (Table 1), with a highly significant overall effect ($P < 0.0001$). At one day of age, all groups showed nearly identical antibody titers (7194.6), indicating a uniform level of maternally derived antibodies at the

beginning of the experiment. These findings indicated comparable levels of maternally derived anti-IBDV antibodies among the groups at the beginning of the experiment. By 10 days of age, antibody titers had declined in entirely groups; nevertheless, the decline varied among groups. Group 1 (G1) maintained the highest antibody titer (3134.66), whereas lower values were recorded in G2 (2034.16), G3 (1842.66), and G4 (1010.33). The control group showed a similarly low level (1130.16), reflecting the normal decline of maternal antibodies in the absence of vaccine-induced reinforcement. At day 15, the highest antibody titer was found in G1 (4207.33) followed by G2 (2083), G3 (2010.83) and G4 (1668) with G5 having a decreased response compared to other groups at 908.80. Therefore, it was possible to conclude that the antibody response developed earlier in G1 than in the other vaccinated groups. At twenty days of age, antibody titers increased considerably. The highest antibody titer was in G2 (10858.66) and G3 (10586), followed by G4 (10115.33) while G1 had an antibody titer of 7608. Conversely, the control group decreased in antibody titer throughout this period resulting in only an antibody titer of 550 which indicates no effective active humoral immunity. At 25 days of age, group one (G1) showed the most significant antibody titer (25406.60) compared to groups two (G2, 18774), three (G3, 15256), or four (G4, 12845.60). Overall, from the results of the serology testing, group one produced a detectable humoral response much earlier than group two (G2) or three (G3) and G1 also had the highest measured antibody titer at 25 days of age while groups G2 and G3 showed very similar intermediate increases in antibody levels on Day 20.

Table 1. ELISA antibody titers against IBDV in broiler chickens vaccinated with different commercial IBD vaccines at different ages

Groups	One day	10 Day	15 Day	20 Day	25 days
Control	7194.6±47.7 Aa	1130.16±1.32Ba	908.80±1.44 Da	550±2.88 Db	------
G1	7194.6±47.7 Aa	3134.66±0.67Ad	4207.33±3.07 Ac	7608±2.76 Cb	25406.60±236Aa
G2	7194.6±47.7 Ab	2034.16±0.94Ac	2083±220 Bc	10858.66±1.93Ab	18774±59 Ba
G3	7194.6±47.7 Ab	1842.66±1.42Ac	2010.83±236 Bc	10586±1.73 Ab	15256±153 Ca
G4	7194.6±47.7 A	1010.33±0.98Bd	1668±283 Cc	10115.33±1.42 Bb	12845.60±129 Da
Calculated P value			<0.0001		
LSD (P<0.05)	7194.6±47.7 Aa		334.5		

Values are expressed as mean ± SD. Means with different capital letters within the same column and different small letters within the same row differ significantly at $P < 0.05$.

***IL-1β* gene expression:** The relative expression of interleukin-1 beta (IL-1β) showed numerical variation among the experimental groups (Table 2). However, these differences were not statistically significant ($P = 0.411$). The control group showed basal expression (1.00), representing the reference level. Among the vaccinated groups, G1 recorded the highest numerical

fold change (3.07 ± 1.62), followed by G2 (1.92 ± 0.74). In contrast, G3 (1.04 ± 0.50) and G4 (1.20 ± 0.51) showed values close to the control group. Although G1 showed a higher mean expression level than the remaining groups, the variation among groups did not reach statistical significance. These findings suggest that vaccination induced only limited variation in IL-1β-mediated inflammatory signalling under the conditions of the present study.

Table 2. Relative expression of interleukin-1 beta (IL-1β) in broiler chickens vaccinated with different commercial infectious bursal disease vaccines

Groups	IL-1β (Fold change)
Control	1±1b
G1	3.07±1.62a
G2	1.92±0.74b
G3	1.04±0.50b
G4	1.20±0.51b
Calculated P value	0.411
LSD(P<0.05)	2.52

Morals are articulated as mean ± SD fold alteration. Means with different letters differ significantly at P < 0.05.

***IFN-γ* gene expression:** In contrast to IL-1β, the relative expression of interferon-gamma (*IFN-γ*) differed significantly among the experimental groups (P = 0.002). The control group showed basal expression (1.00). Whereas, G1 exhibited a marked increase in *IFN-γ* fold change, reaching 7.96 ± 2.57, which was significantly higher than all other groups (Table 3). A moderate increase was observed in G3 (3.08 ± 0.68), while G2 (1.37 ± 0.44) and G4 (1.11 ± 0.30) remained close to the control level. The statistical grouping indicated that G1 was distinctly separated from all other groups, confirming that it induced the strongest cellular immune activation among the tested vaccines.

Table 3. Relation appearance of interferon-gamma (*IFN-γ*) in broiler chickens vaccinated with diverse commercial transmittable bursal sickness vaccines

Groups	Interferon gamma (Fold change)
Control	1±1b
G1	7.96±2.57a
G2	1.37±0.44b
G3	3.08±0.68b
G4	1.11±0.30b
Calculated P value	0.002
LSD(P<0.05)	3.54

Values are expressed as mean ± SD fold change. Means with different letters differ significantly at P < 0.05.

Molecular confirmation of the challenge isolate by conventional RT-PCR: Infectious bursal disease virus was found in samples from the field; RT-PCT testing of the *VP2* gene was conducted. Agarose gel electrophoresis of PCR products indicated bands at the expected size of

253 bp (Figure 1). Thus, it confirmed the specificity of the primers and the PCR protocol used. Through amplification of the observed patterns, verification of the molecular identity of the isolate as Infectious Bursal Disease Virus (IBDV) were accomplished and its use as the confirmatory leadoff challenge virus in this experimental model was supported. Positive amplicons (PCR products) from all of the analyzed samples were obtained and consistent results were obtained indicating the reliability of this RT-PCR detection protocol to local field isolates.

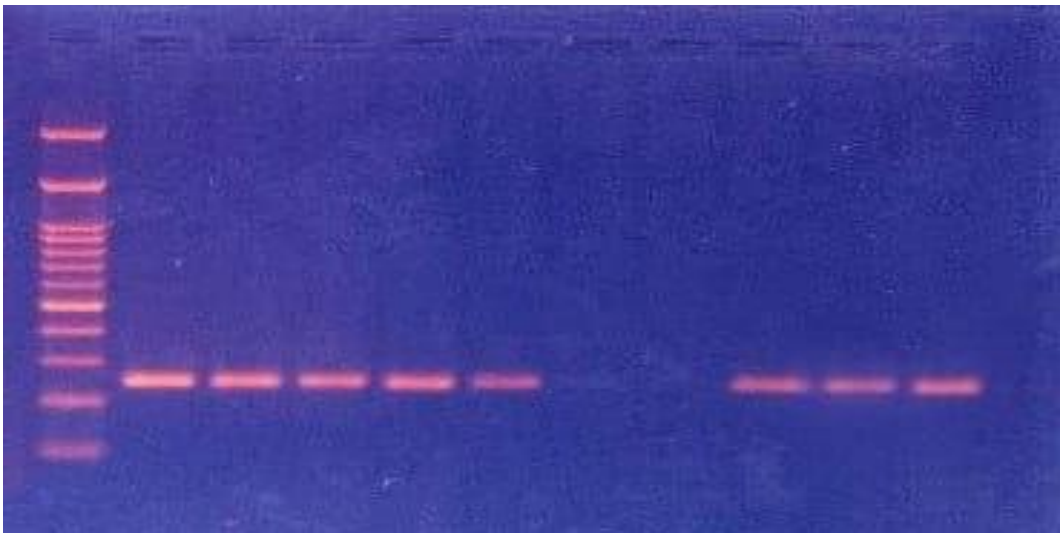

Figure 1. Agarose gel electrophoresis of conventional RT-PCR creations augmented as of characteristic bursal samples directing the *VP2* gene of infectious bursal sickness virus (IBDV). Lane M: DNA molecular size marker; sample lanes display positive amplicons at the predictable size of 253 bp

Integrated interpretation of the immunological findings: Serological and cytokine evaluations (conducted together) provided clear distinctions regarding the performance of the individual vaccine types/brands. Group 1 (G1) exhibited the earliest antibody response as well as the greatest number of responders producing *IFN-γ* at both 10 and 15 days post-vaccination, indicating that there was a significant amount of cellular immune activation in response to vaccination in this group. Antibody levels at 20 days post-vaccination were significantly greater in both Group 2 (G2) and Group 3 (G3) than in G1, but G1 regained the highest average final antibody titre (all groups together) by 25 days of age. In contrast, the profile for *IL-1β* was fairly consistent. There was no clear ability to differentiate between groups based on *IL-1β* levels. Overall, the immunological profile indicates that Group One (G1) had the most prompt early immune activation; whereas, Groups Two (G2) and Three (G3) had delayed yet larger humoral responses. Collectively, these results demonstrate that the three vaccines elicited different patterns of magnitude and timing for both humoral and cellular immune responses.

Discussion

The current study showed the difference in immunogenicity of various commercial IBD vaccines in addition to the magnitude of the immune response and time at which the response

occurred. This distinction is very important in broiler production because IBDV affects birds early in life before their immune systems are fully developed and maternal antibody interference will greatly influence how well the vaccine works. As such, early immunogenicity is a crucial factor to consider when comparing IBD vaccines (Dey et al., 2019; Alkie & Rautenschlein, 2016). Results of the current research indicate that all groups had similar antibody titers at 1 day of age which suggests that the level of maternal derived antibodies was also equal at the start of this study. Therefore, it would appear that the differences observed between groups following this point were likely a result of how well each of the different vaccines performed and not due to unequal levels of passive immunity at the conclusion of the trial. The decrease that occurred within the control group is consistent with how maternal antibodies naturally decrease over time in chickens raised for meat production. In contrast, the random variability between vaccinated groups reflects the ability of the vaccines being tested to establish some form of active immunity despite having had maternal antibodies present (Naqi et al., 1983; Solano et al., 1986). The results of ELISA analysis confirmed that group 1 was the vaccine that had generated the earliest increase in detectable anti-IBDV antibody titers among the vaccinated groups due to its ability to produce the highest antibody titres by the 10- and 15-day time points. Therefore, this finding is among the most valuable of the outcomes measured during this trial, as it fulfilled the primary goal of this study which was to compare immunologically the vaccines against each other, and determine which vaccine would induce protection in birds in the shortest time possible. While Group 2 and Group 3 were able to elicit considerably higher antibody responses at the 20-day time period, Group 1 regained superiority at 25 days of age, and ended up producing the highest final antibody response. This finding indicates that Group 1's initial advantage was not limited to an early priming effect, but that group 1 also had a greater ability to produce a stronger late humoral response. Other similar comparative studies have shown significant differences between various commercial IBD vaccines in regard to both the speed of seroconversion, and the timing with which effective immunity is achieved in broiler poultry (Hamad et al., 2020, Legese et al., 2022). While the *IL-1β* results provide for a useful, albeit limited, augmentation to the interpretation of the immune profile, the fact that Group 1 had the highest fold change but was not statistically significant implies that the tested vaccines did not produce significantly different pro-inflammatory responses under these experimental circumstances - potentially favorable from a biological point of view, as an effective vaccine should elicit immune recognition without producing significant levels of inflammatory stress or unwarranted immunopathological burden. The superior early profile for Group 1 was therefore not associated with specific evidence of more than expected levels of inflammatory activation (Qin & Zheng, 2017; Liu et al., 2010). In this study, the findings show clearly defined differences between the groups based on *IFN-γ* expression, with Group 1 exhibiting a significantly greater *IFN-γ* response than any other group suggesting the other groups had lower levels of early cellular immune activation compared to Group 1. This finding is clinically relevant because *IFN-γ* is an important cytokine involved in antiviral and cell-mediated immune responses and has been well established as critical to host immune defense during IBDV infection. Previous studies identified specific cytokine profiles (including those related to IFN) that are affected by viral virulence and host genetics and thus will

help differentiate between clinical outcomes following viral infection/vaccination (Raj et al., 2011; Qin & Zheng, 2017). Therefore, given the large upregulation of *IFN-γ* in Group 1, we can conclude that the vaccination tested in Group 1 led to the earliest/most coordinated priming of the immune response among those tested in this study. Considering the serological and cytokine data together reveals a stronger overall pattern than with the serological information alone. Group 1 showed both an early humoral activation and an early cellular activation by having higher levels of *IFN-γ*, but *IL-1β* was elevated only numerically and statistically controlled for. Therefore, the coordinated response for Group 1 demonstrates that the superiority of this group was due to both humoral and cellular immune activation rather than to one endpoint. In contrast, Group 2 and 3 exhibited an antibody response that was delayed, but substantial; and Group 4 exhibited a much lesser degree of responses overall. Previous evaluations of vaccines for IBD have also noted that to properly compare between the various vaccines for IBD one must utilize immune kinetics and multiparameter responses instead of using a single serological result later on (Gewaily et al., 2024; Hamad et al., 2020). The use of conventional RT-PCR to confirm the molecular characterisation of the challenge isolate using the *VP2* gene adds further support to the experimental model. As expected, detection of a 253-bp band indicated that the field isolate used as a challenge was IBDV-positive. The molecular confirmation is significant because the *VP2* gene is still among the most clinically useful and frequently utilised in both the diagnostic setting of IBDV and as an investigative tool in molecular characterisation of IBDV. Molecular studies have used *VP2*-based amplification to repeatedly demonstrate the diagnostic and biologic usefulness of *VP2* amplification for the confirmation of field isolates as well as for strain-related assessments (Sharma et al., 2005; Dey et al., 2019). The results obtained through this analysis clearly demonstrate that immunologic profiles of the various vaccine groups ranked Group 1 as having the most favorable early immunologic profile, demonstrated by rapid development of antibodies, the highest relative expression of *IFN-γ* mRNA, and the highest titers of antibody at the end of the study period. These findings indicate differences among the vaccines in the kinetics and magnitude of the measured immune responses. Whether these differences translate into superior protection requires confirmation using standardized post-challenge clinical, pathological, and virological endpoints. From a practical standpoint, this information supports the use of vaccines that can induce early immune priming for the production of broilers at risk of IBDV infection (Alkie & Rautenschlein, 2016; Wang et al., 2025).

Conclusion: The current study showed that the commercial vaccines for infectious bursal disease had varying degrees of immunogenicity when tested broiler chickens. While the vaccinated chickens all had immunologic responses, each had significantly different onset timings and magnitudes of their immunologic responses. Group 1 had the earliest onset of antibody response (the highest antibody titers) at Days 10 and 15 after being vaccinated; therefore, Group 1 was the most significant cellular immune response activator (the relative *IFN-γ* transcript abundance was significantly higher). Moreover, Group 1 regained its superiority at Day 25 (the highest final antibody titer), compared to the other vaccines. No significant differences were observed in the expression of *IL-1β* among groups, which suggests that Group 1's superior early response was due to having the highest antibody titer produced without an excessive amount of

inflammation. Additionally, the molecular confirmation of the local field isolate (*VP2*-targeted conventional RT-PCR) adds support to the experimental model's validity. Overall, commercial IBD vaccines exhibited distinct temporal patterns of immune activation, with G1 showing an earlier and more pronounced measured immune response, whereas G2 and G3 showed delayed but substantial humoral responses.

Authors contribution

Both authors confirm contribution to the paper equally.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical considerations

The experimental procedures were approved by the Institutional Animal Ethics Committee and conducted at the Veterinary Medicine College, Al-Qasim Green University, Iraq (No. 111-2025).

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No funding was received.

Conflict of interest

The authors have no conflict to declare.

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ارزیابی مقایسه‌ای پاسخ‌های هومورال و سیتوکینی اولیه القاشده توسط چهار واکسن تجاری بیماری بورس عفونی در جوجه‌های گوشتی

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چکیده

هدف: ویروس بیماری بورس عفونی (IBDV) یک عامل بیماری‌زای ویروسی مهم در جوجه‌های گوشتی است، زیرا تمایل بافتی آن به لنفوسیت‌های B نابالغ موجود در بورس فابریسیوس می‌تواند موجب سرکوب سیستم ایمنی شود. مطالعه حاضر پاسخ‌های ایمنی اولیه ایجادشده توسط چهار واکسن تجاری بیماری بورس عفونی (IBD) را در جوجه‌های گوشتی، از طریق تحریک پاسخ‌های سرولوژیک و سیتوکینی، مقایسه کرد.

مواد و روش‌ها: در مجموع، ۲۵۰ قطعه جوجه گوشتی یک‌روزه نژاد Ross 308 به‌صورت تصادفی به پنج گروه ۵۰ قطعه‌ای تقسیم شدند و در گروه‌های درمانی مربوطه قرار گرفتند. چهار گروه، هرکدام یکی از چهار نوع واکسن تجاری را دریافت کردند (G1-G4) و گروه پنجم به‌عنوان گروه کنترل (G5) در نظر گرفته شد و واکسن دریافت نکرد. پاسخ آنتی‌بادی سیستم ایمنی با استفاده از ELISA غیرمستقیم در روزهای ۱، ۱۰، ۱۵، ۲۰ و ۲۵ ارزیابی شد. همچنین، RT-PCR کمی (qRT-PCR) در روزهای ۱ تا ۲۵ برای اندازه‌گیری بیان نسبی *IL-1β* و *IFN-γ* انجام شد. علاوه بر این، جدایه میدانی مورد استفاده در مطالعه چالش، با استفاده از RT-PCR معمولی و هدف قرار دادن ژن *VP2*، از نظر وجود IBDV شناسایی شد.

نتایج: تیتر آنتی‌بادی مادری در همه گروه‌ها در سن یک‌روزگی مشابه بود. در سنین ۱۰ و ۱۵ روزگی، گروه ۱ بالاترین تیتر آنتی‌بادی را نشان داد و در این زمان‌ها دارای پاسخ هومورال اولیه بیشتری بود و در روز ۲۵ به بالاترین تیتر نهایی نزدیک شد. هر دو گروه ۲ و ۳ در سن ۲۰ روزگی تیتر آنتی‌بادی متوسطی داشتند که از تیتر گروه ۱ بیشتر بود. سطح بیان *IL-1β* بین گروه‌ها تفاوت معنی‌داری نداشت، اما گروه ۱ بیشترین افزایش بیان را بر اساس تعداد افراد واردشده در تحلیل نشان داد. در مقابل، سطح بیان *IFN-γ* بین

گروه‌ها تفاوت معنی‌داری داشت. به‌طور کلی، واکسن‌های تجاری IBD مورد بررسی از نظر زمان و شدت پاسخ‌های ایمنی ایجادشده با یکدیگر تفاوت داشتند. در میان سه گروه مورد بررسی، گروه ۱ مطلوب‌ترین الگوی ایمنی‌زایی اولیه را داشت؛ زیرا موجب ایجاد زودتر آنتی‌بادی هومورال، بیان قوی‌تر *IFN-γ* و بالاترین تیتر نهایی IgY شد.

نتیجه‌گیری: یافته‌ها نشان می‌دهند که استفاده از سینتیک یکپارچه پاسخ‌های ایمنی می‌تواند به‌عنوان روشی برای تولید داده‌های مقایسه‌ای درباره ایمنی‌زایی واکسن‌های IBD در جمعیت جوجه‌های گوشتی مورد استفاده قرار گیرد.

کلمات کلیدی: ایمنی‌زایی واکسن، بیماری گامبورو، ژن *VP2*، IL-1β، *IFN-γ*

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