Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2025; 6(2): 205-208
Received: 04-07-2025
Accepted: 09-08-2025

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Efficacy of var 206 vaccination against infectious bronchitis virus in broiler chickens in Babylon, Iraq

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DOI: https://www.doi.org/10.33545/27080013.2025.v6.i2c.255

Abstract
The production of chicken is still severely hampered by the infectious bronchitis virus (IBV), which causes respiratory illnesses, decreased productivity, and large losses. Despite routine immunization, outbreaks continue to occur often in Iraq. Under field conditions in Babylon Province, this study examined the effectiveness of immunization programs in broiler chickens, with a particular emphasis on the Var206 vaccine. Five groups of 260 Ross 308 chicks each were formed. One group was used as an unvaccinated control, and the other four groups got various combinations of the Newcastle disease virus (NDV) and IBV vaccinations. ELISA was used to measure antibody titers at various ages. The findings demonstrated that the strongest and most reliable immune response against IBV was generated by two doses of Var206.Programs such as Clone 30 and LaSota had the highest levels of NDV immunity. These findings emphasize the practical importance of Var206 as a homologous vaccine and the need to adapt vaccination strategies to local field strains in Iraq.

Keywords: Infectious bronchitis (IBV), maternal immunity, humoral immunity, var 206 vaccine, broiler chickens, Iraq

Introduction
First identified in the United States in 1931, infectious bronchitis (IB) is one of the oldest known viral illnesses of chickens then, despite decades of research and vaccine efforts, it has emerged as a significant global hazard to the chicken industry, with recurrent outbreaks (Khan *et al*., 2023) [6]. It has an economic impact on the kidneys, reproductive organs, and respiratory system. Depending on secondary infection by bacterial infections, egg production might decrease by 50% or more, morbidity is 100%, and mortality can reach 30% (Phuntsho, 2022) [10]. Clinically, IB is mostly a respiratory condition that results in tracheal rales, nasal discharge, coughing, and sneezing.
Depending on the virus strain and host variables, it can spread to other organs in extreme situations, resulting in reproductive problems and nephritis (Hassan, 2022) [5]. These issues exacerbate economic losses by making people more vulnerable to subsequent diseases. Infectious bronchitis virus (IBV), the causal agent, is a member of the family Coronaviridae and genus Gammacoronavirus. Rapid genetic evolution is a characteristic of this positive-sense RNA virus, especially in the spike (S) glycoprotein gene. Numerous serotypes and genotypes have emerged as a result of this genetic heterogeneity, and they frequently exhibit poor cross-protection with conventional vaccines (Mo, 2018) [7]. IB has been documented in Iraq for a number of decades, and outbreaks in broiler and layer flocks are common.
Due in major part to the spread of many variations and inadequate biosecurity measures, the disease continues to be endemic even with regular vaccination campaigns (Seger *et al*., 2016) [11]. Therefore, it is crucial to continuously monitor circulating strains and assess local vaccination programs in order to lessen the impact of IBV on the country's chicken industry (Guzmán & Hidalgo, 2020) [4].

Materials and Methods
The chicks and Housing: Al-Nasr Hatchery, Babylon Governorate, provided 260 one-day-old (Ross 308 broiler chicks) in total. Similar management conditions applied to the housing of the chicks. Fresh drinking water and a typical commercial broiler meal were given to the birds. Standard broiler management procedures were followed to maintain thse temperature, lighting, and ventilation.

Maternal Antibody by Enzyme-Linked Immunosorbent Assay (ELISA)

Ten one-day-old chicks were chosen at random to use ELISA to measure maternally derived antibodies (MDA). A heart puncture was used to get blood, which was then examined at the Veterinary Hospital Laboratory in Babylon. Antibodies against IBV, NDV, and H9 were detected sequentially using ELISA kits: ID Screen® Infectious Bronchitis Indirect 2.0, ELISA Kit ID Screen® Newcastle Disease Indirect Conventional Vaccines, and ELISA Kit ID Screen® Influenza H9 Indirect Innovative Diagnostics/France, in accordance with the manufacturer's instructions.

Vaccination programs

The remaining 250 chicks were first vaccinated at the hatchery with inactivated [QVAC ND (G7) + H9/China] by subcutaneous route of vaccine. They were then divided into five groups (A, B, C, D and E, 50 birds each) and treated as follows:

- **Group A:** Day 1 - Nobilis IBV Ma5 + NDV Clone 30 (eye-drop); Day 10 - NDV Clone 30 (coarse spray); Day 14 - IBV Var 206 (drinking water); Day 20 - NDV LaSota (drinking water).
- **Group B:** Day 1 - CHB (NDV Clone 30, IBV H120 and IBV 28/86) + IBV 4/91 (eye-drop); Day 10 - NDV Clone 30 (coarse spray); Day 14 - IBV Var 206 (drinking water); Day 20 - NDV LaSota (drinking water).
- **Group C:** Day 1 - IBV Var 206 (eye-drop); Day 14 - IBV Var 206 (spray).
- **Group D:** Day 1 - IBV Ma5 (eye-drop); Day 14 - IBV 4/91 (drinking water).

  Group E: control without vaccination.

Vaccines Handling

Every vaccination was given in accordance with the guidelines provided by the manufacturer. A calibrated dropper was used to administer eye drops (~30 µL per dosage, 30 mL solvent/1000 doses). On day 10, a coarse spray (droplets ranging from 250 to 300 µm) was used to administer the immunization. Vaccines for drinking water were made in cold, chlorine-free water. Vaccines were used right away after preparation and thrown out two hours later.

Monitoring of Humoral Immunity

At 7, 14, 20, and 26 days of age, wing vein blood samples were taken. ELISA was used to determine antibody titers against IBV, NDV, and H9 in the serum. To compare the immunological responses of the various groups, mean titers were computed.

Statistical Analysis

Software called SPSS (version 16.0; IBM Corp., USA) was used to evaluate the data. One-way ANOVA was used to evaluate group differences.

Results and Discussion

Maternal Immunity

The ELISA results on Day 1 confirmed effective transfer from breeders with high maternally generated antibody titers (19,595) for H9, (13,676) for NDV, and (10,800) for IBV. Strong maternal transfer following inactivated vaccination in breeders is reflected in the increased H9 baseline (Pan *et al*., 2022) [9s].

Post -vaccination humoral immune response against IBV, NDV

IBV antibody titers at day 7 varied from 5875.6 in group C to 7324.4 in group A. All groups experienced a decrease by day 14, with group D remaining at the highest level (6733) and group B reaching the lowest level (2899).Titers rose significantly in the vaccinated groups at day 21, especially in groups C (13116) and D (9806).The control group E, on the other hand, only displayed moderate levels (9379), indicating that there was no booster effect. On day 26, groups A, B, and the control E showed decreased levels (6833-7382), whereas group C maintained the highest response (10000) and group D showed sustained titers (8377). (Fig. 1).

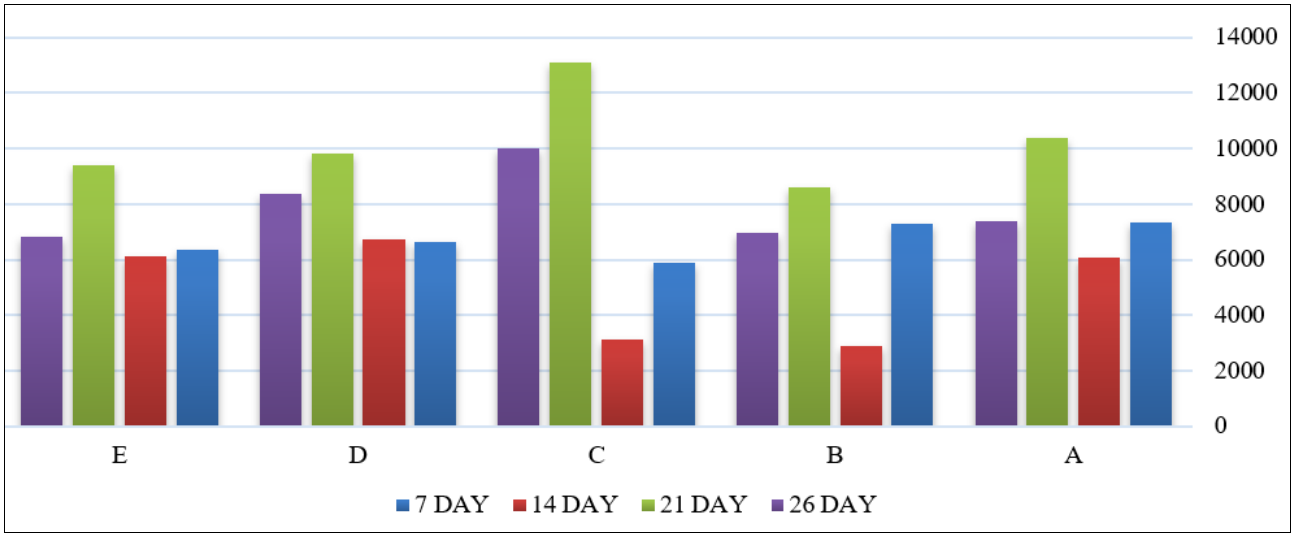

Fig 1: Showing IBV Mean ELISA Antibody Titers of the experimental groups across Different Days

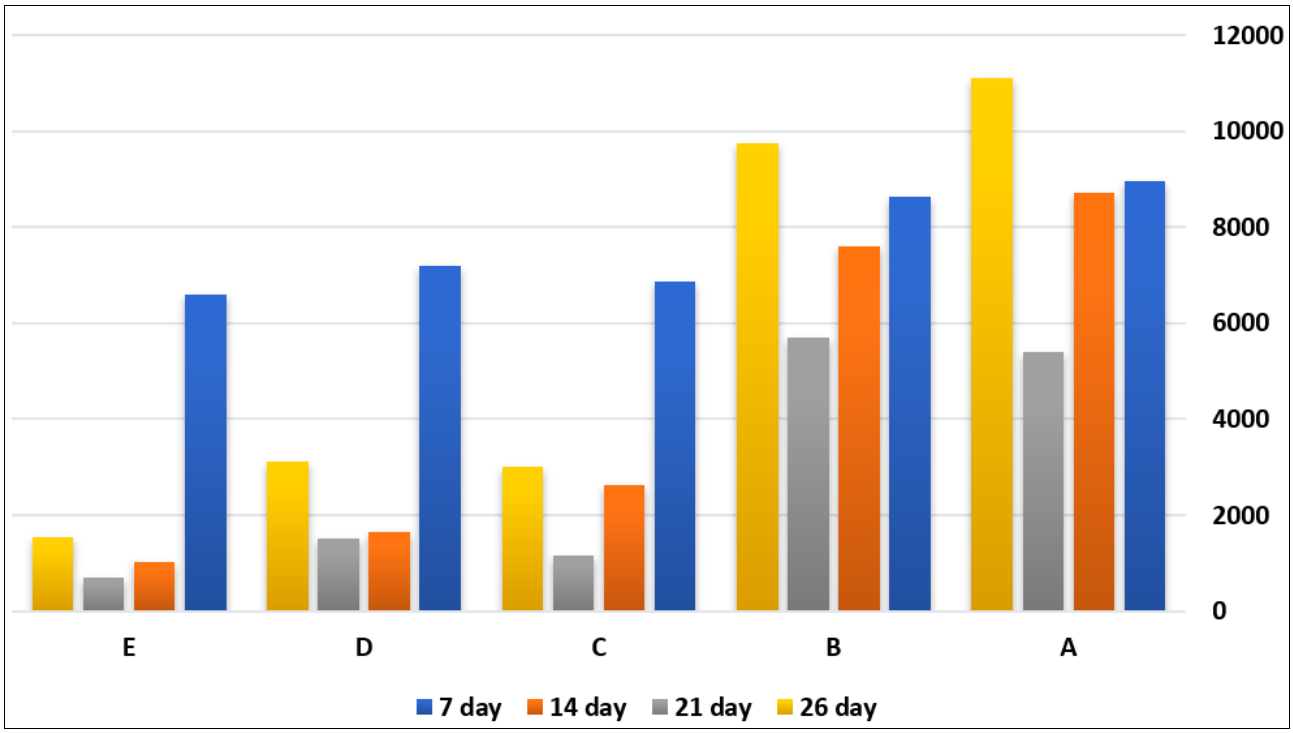

Fig 2: Showing NDV mean ELISA Antibodiy titer of the experimental Groups across Different days

The benefit of homologous prime-boost vaccination in producing robust and long-lasting humoral immunity is confirmed by the better response in group C, which received two doses of Var206 (Al-Qaisi & Abumsimir, 2023) [1]. Groups A and B were less persistent than Group D, which also showed strong persistence. In line with previous IBV research, the control group E only maintained moderate titers, highlighting the fact that maternal antibodies or natural exposure are insufficient for long-term protection (Bhuiyan *et al*., 2021) [2].

However, NDV data (Figure 2) revealed that all vaccination groups experienced an early spike at day 7, with groups A and B receiving Clone 30 at hatch exhibiting the highest levels. By day 14, there was a slight decrease, and by day 21, there was a noticeable rise, particularly in group A following the LaSota booster. Groups A and B continued to have high titers on day 26, but groups C and D responded moderately, and the control group stayed at the bottom. According to these results, compared to single-strain schedules, heterologous vaccination programs (Clone 30 prime followed by LaSota boost) produce stronger and longer-lasting immunity. This is consistent with new research showing that combined NDV vaccines increase the breadth and duration of protection in broilers (Dewidar *et al*., 2022) [3]. The observed mid-cycle decline followed by recovery reflects the natural kinetics of antibody development and highlights the importance of timely booster administration (Nayak *et al*., 2023) [8].

Conclusion

Our results demonstrate that the most effective and durable protection against IBV in broilers is provided by two doses of the Var206 vaccination. Stronger NDV protection was supported by programs that combined Clone 30 with LaSota. Early and appropriately tailored vaccination techniques are still essential to manage IBV and protect Iraqi poultry output, given the sharp reduction in maternal antibodies.

Conflict of Interest

The authors declare no conflict of interest.

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