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Immunological and histopathological evaluation of the seventh day Intermediate Plus Tissue Culture live attenuated IBD vaccine in commercial broiler chickens

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ABSTRACT

Infectious Bursal Disease (IBD) causes significant challenges to the poultry industry, particularly with the emergence of novel variant Infectious Bursal Disease virus (IBDV) strains. Effective vaccination program is required for controlling this disease, but the impact of such vaccines on the bursa of Fabricius, the main immune organ, remains a concern. This study had been conducted for immunological evaluation of early vaccination with Intermediate plus tissue culture (INP-TC) IBD vaccine (Bursipharm®) and to assess the impact of this vaccine on the bursa of Fabricius in broiler chickens. No significant differences were observed in body weight and feed conversion ratio between the vaccinated and control groups. The relative weight of the bursa and spleen did not differ significantly, and the bursa/body index indicated no significant atrophy on the bursa of Fabricius in vaccinated birds. Immunologically, IBDV antibody titers were significantly higher in the vaccinated group, whereas Newcastle disease virus (NDV) antibody levels showed no significant differences. Histopathological lesion score in bursa of Fabricius showed mild to moderate lesions in the vaccinated group without causing any pathological atrophy throughout the experiment. Using the intermediate plus IBDV tissue culture origin vaccine (Bursipharm®) at 7 days and the second dose at 14 days induced an effective immune response against Gumboro disease with controllable effects on the bursa of Fabricius. The vaccine can overcome maternal derived antibodies (MDA) and initiate an immune response as early as possible, which becomes beneficial in combating novel variant strain of IBDV and preventing the replication of this strain in the bursa of Fabricius at an early age.

Introduction

The chicken industry suffers huge losses owing to the immunosuppressive disease known as infectious bursal disease (IBD). IBD infects young chickens and weakens their immune system. It is caused by a birnavirus and mostly affects the bursa of Fabricius, a chief organ in poultry for the growth of their immune systems (Tahir and Alsayeqh, 2024; Ziaf *et al*., 2024)

Infectious bursal disease virus (IBDV) is a highly contagious, positive sense, single-stranded, and RNA-based. Multiple IBDV serotypes are often detected co-circulating on farms, with little cross-protection from the vaccination strain against unrelated field strains (Tahir and Alsayeqh, 2024).

Over the past 30 years, this disease has been well controlled based on the scientific strategy of vaccination and the strict biosafety methods. However, the appearance of novel variant IBDV strain in recent years made a new threat to the chicken industry (Leng *et al*., 2023).

A major epidemiological shift has been observed in Egypt with the detection of those novel variant IBDV strains (genotype A2dB1b) together with the previously prevalent very virulent strains. These variant strains infect at an early age, before 14 days, and cause severe bursal atrophy and immunosuppression (Legnardi *et al*., 2023), that result in increased morbidity and mortality rates, which directly threaten the health and productivity of poultry flocks. The presence of novel variant IBDV creates questions about the efficacy of the current vaccinations, which were created to guard against previous IBDV strains. This is especially crucial because there's a chance that novel variant IBDV has genetic variations that let it avoid the immunological reaction that these vaccinations produce, which could result in health problems and financial losses in the poultry flock (Salaheldin *et al*., 2024). So, the development of novel and effective vaccines with minimal or no damage to the bursa of Fabricius is in urgent demand.

According to Leng *et al*. (2023), commercial yellow-feathered broilers and Specific Pathogen-Free (SPF) chickens were protected against novel variant strains by the attenuated live vaccination (2512 strain). Researchers discovered that 2512 creates large levels of antibodies against IBDV, restricts the viral development of novel variant strains via placeholder effect, but causes severe atrophy of the bursa of Fabricius in SPF hens and commercial yellow-feathered broilers.

In order to prevent this, it may become important to fight with less attenuated intermediate plus or hot vaccines by considering farms where IBD outbreaks occur as endemic. In such cases, the degree of bursal damage, the time it takes for lesions to heal, and the length of the rearing period should all be considered when choosing a vaccine (Ateş *et al*., 2022).

Presently, there are two methods used to manufacture commercial IBD vaccines: cell-based vaccines or egg-based vaccinations. It is important to note that, for a number of reasons, more contemporary cell culture-based techniques are preferred than earlier egg-based techniques (Butt *et al*., 2015).

Using strain G6, which was produced in tissue culture to judge the safety and effectiveness of a live, intermediate plus vaccine against infectious bursal disease, revealed that G6 strain-based vaccine showed good safety and efficacy profiles, satisfying European Pharmacopoeia restrictions (Huić Babić *et al*., 2021).

Another study compared CEF cell-based and DF-1 cell line-adapted Gumboro vaccines and found that DF-1 cell-line can be considered an affordable and convenient alternative to the CEF-based approach (Workineh *et al*., 2022).

The IBDV vaccine strain was determined to be immunogenic, well-adapted on Vero cells, and to successfully protect chicks against challenge with the circulating field isolate by inducing antibody formation. Therefore, in order to overcome the drawbacks associated with the

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use of CEF cells and vaccinate the chick population against the circulating IBDV infection, it is advised to create an IBD vaccine employing Vero cell culture on an industrial scale (Kebede *et al*., 2021).

Bursipharm® is an attenuated live vaccine, INP- IBD strain 2512 tissue culture origin, Pharmagal. Bio, Slovac Republic, the recommended vaccination scheme by the manufacture company is Vaccination of Chicks on 7th day of age, and revaccination on 14 days after primo vaccination.

Furthermore, around one week of age, the maternally derived antibodies (MDA) of IBD showed a downward trend before abruptly declining to a non-protective level (Thomrongsuwannakij *et al*., 2021). Furthermore, Meher *et al*. (2022) proposed that the protective level of the IBDV MDA titer can last for as long as one week following hatching. Thus, immunizations soon following this period may be beneficial.

The aim of this study was to evaluate the histopathological and immunological effects of early vaccination (at 7 days followed by a second dose at 14 day) with an intermediate plus Infectious Bursal Disease Virus tissue culture origin vaccine (Bursipharm®) on the bursa of Fabricius in broilers.

Materials and methods

IBDV and NDV Vaccines

(Bursipharm®), attenuated live vaccine, INP- IBD strain 2512 tissue culture origin (Batch number: 16062201, manufacture date: 06-2022, and expire date: 06-2024), Pharmagal. Bio, Slovac Republic (G2 only, days 7th and 14th, intra-ocular).
MEVAC® ND HB1, lyophilized live attenuated ND vaccine (Batch number: 2206010401, manufacture date: 06-2022, and expire date: 06-2024) (All chicks, day 0, intra-ocular).
MEVACTM ND7 PLUS, inactivated bivalent recombinant vaccine (Batch number: 220602010, manufacture date: 06-2022, and expire date: 06-2024) (All chicks, day 5th, intramuscular).
MEVAC® ND LaSota, lyophilized live attenuated ND vaccine (Batch number: 2305020401, manufacture date: 05-2023, and expire date: 05-2025) (All chicks, days 10th and 20th, intra-ocular).

Experimental study

Animals

Following the OIE standards for the use of animals in research in accordance with ARRIVE guidelines, a total of one hundred seventy-five broiler chicks were received at one day old and housed in experimental units of the Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Assiut University. These facilities were approved by the National Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. Under No. 06 / 2024 / 0240. Twenty-five chicks were euthanized humanely immediately upon arrival for a serological test. After seven days, the remaining chicks were divided into two equal groups of 75 each. Chickens of both groups were further subdivided into three equal replicates, each containing twenty-five, which kept in isolators with strict isolation measures. G1 was used as a control. While birds in group G2 were vaccinated with (INP-TC) IBDV vaccine (Bursipharm®) at 7 days of age, and a second vaccination was done at 14 days of age. Vaccines were administered via the recommended intraocular route. For both groups, the Newcastle disease vaccination was the same.

Sampling and data collection

Samples (Serum samples for immune response assessment and tissue samples for histopathological examination) were collected weekly from both groups at the ages of 14, 21, and 35 days. Every week, fifteen chicks from each group (5 from each replicate) were euthanized humanely.

Body weight and feed conversion ratio (FCR)

Body weights (gram) of the chicks were recorded using a digital scale at 0, 7, 14, 21, 28 and 35 days of the experiment.
The FCR was calculated by dividing the total feed intake on body weight gain over the specified period (Shehab, 2008).

Relative organ weights and Bursa to body weight index

Bursa of Fabricius and spleen were weighed, and their relative weights were calculated according to Verma *et al*. (2004) as the following formula:
Organ weight (g)/live body weight (g) ×100
Bursa/body index (BB index) = BB ratio of infected (or vaccinated) birds/ BB ratio of the controls (Raji *et al*., 2017).
Standards of the BB index used in classifying IBD viruses, or conventional live IBD vaccines according to Raji *et al*. (2017).

BB index interpretations

>0.7= Physiological variability = No atrophy.
0.3-0.7= Relative and transient atrophy.
<0.3= Strong atrophy.

Immune Response Assessment

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Sera collected at day 1 were tested to determine MDA against IBD, then humeral immune response induced by vaccination was measured at 7, 14, 21, and 35 days. Serological titration of IBD-antibodies was performed using commercial indirect ELISA kit (ID Vet, France) in accordance with the protocol specified by the supplier.

Hemagglutination inhibition (HI) test

This was applied for the measuring of antibody titers against Newcastle disease at 21 and 35 days of age using 4 HA unit according to OIE (2012).

Histopathological examination

Fresh specimens of bursa from 15 birds at 14, 21, and 35 days of age were collected and fixed in 10% neutral-buffered formalin. The tissues were dehydrated in a graded alcohol series, cleared with methyl benzoate, embedded in paraffin wax, sectioned at 4- mm thickness, stained with hematoxylin and eosin for histopathological examination by light microscopy (Olympus CX31, Japan), and photographed using a digital camera (Olympus Camedia C-5060, Japan) (Suvarna *et al*., 2019).

Bursa lesion score

According to Hussein *et al*. (2018), bursal lesions were scored into 6 grades as follows:
0= Normal.
1= Mild degeneration and necrosis in the medullary area of lymphoid follicles.
2= Mild to moderate degeneration and necrosis in lymphoid cells of follicles, particularly in the medulla.
3= Moderate necrosis affecting both cortex and medulla.
4= Moderate to severe depletion of lymphoid cells in follicles. Aggregates of lymphocytes were found in the cortex, while the medulla had cysts and necrotic cells.
5= Follicles displayed moderate to severe atrophy with cellular degeneration and necrosis in both cortex and medulla.

Statistical Analysis

Differences between the 2 groups were analyzed by using an independent sample t test. Statistical analysis was performed using the statistical software package SPSS for Windows (version 2016; SPSS Inc., Chicago, IL, USA). Statistical significance between mean values was set at $P < 0.05$.

Results

Performance parameters

There was no significant difference in body weight between the vaccinated group (G2) and the control group (G1) during the whole experimental period (Fig. 1). On the other hand, there was a significant increase in feed conversion ratio in the vaccinated group only at days 14th of the experiment when compared with the control group (Fig. 2).

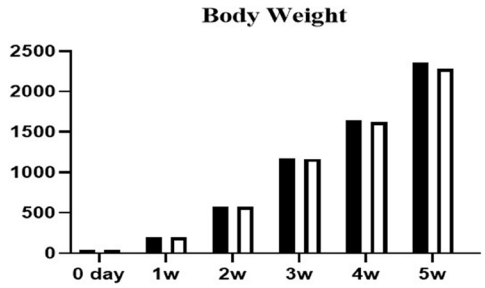

Fig. 1. Effect of INP-TC vaccine (Bursipharm®) on body weight of the broiler chickens.

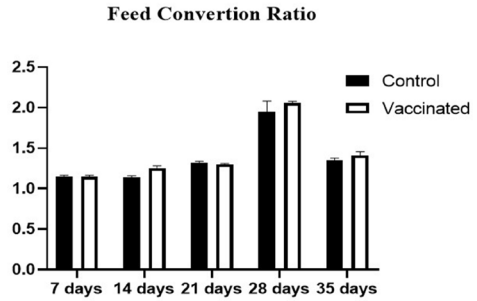

Fig. 2. Effect of INP-TC vaccine (Bursipharm®) on feed conversion ratio in experimental groups.

Immunological parameters

Results shown in Fig. 3, provided the effect of INP-TC IBDV vaccine (Bursipharm®) on the relative weight of bursa of Fabricius (g), it was found that there were no significant differences between the vaccinated group (G2) and control group (G1) during the whole experimental period. Fig. 4 illustrates the bursa/body index, which revealed that vaccination didn't cause atrophy on the bursa of Fabricius. Furthermore, there were no significant differences in the relative weights of spleen during the experiment (Fig. 5).

The obtained data in Fig. 6 indicated that Bursipharm® vaccine didn't induce any significant difference in ND antibody titers within the two groups. There was a highly significant increase ($p < 0.0001$) in IBDV antibody titer in the vaccinated group (G2) when compared with the non-vaccinated group (G1) at 14, 21, 28 and 35 days (Fig. 7).

The mean MDA titer of IBD was 8026±354.5, which was observed on day 0 and then reduced to 4047±258.5 at the age of 7 days. At 14, 21, 28 and 35 days G1 mean titers of IBD were 1073±57.83, 259.4±9.74, 61.60±2.82 and 14.8±0.86, respectively, while G2 mean titers of IBD for 14, 21, 28 and 35 days were 2588±65.81, 3450±196.3, 4559±165.3 and5478±172.7, respectively. This means that from age of 14 days, G2 showed an upward tendency of mean antibody titer and continued up to the age of 35 days (Fig. 7).

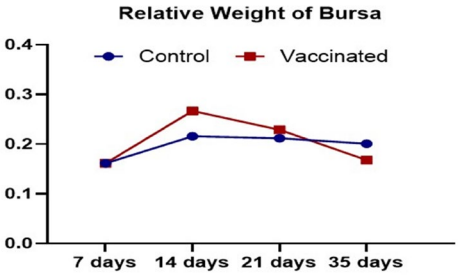

Fig. 3. Relative weight of bursa of Fabricius.

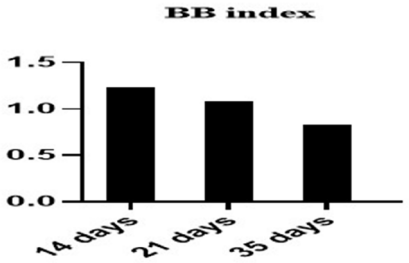

Fig. 4. Mean values of Bursa/Body Index.

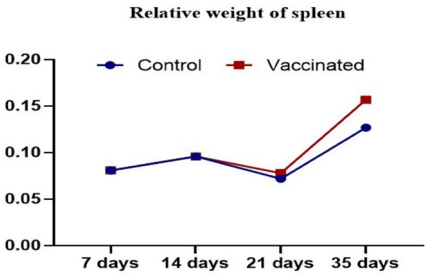

Fig. 5. Relative weight of spleen (g) in experimental groups.

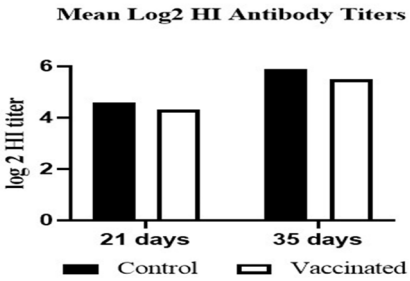

Fig. 6. ND antibody titer in commercial broilers at 21 and 35 days of age by HI test

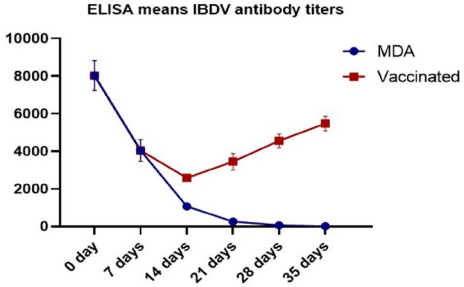

Fig. 7. ELISA means IBDV antibody titer in experimental groups.

Histopathological findings

Microscopic examination of H&E stained tissue sections from the bursae of the sacrificed control birds (G1) revealed histological features of normal lymphoid follicles at different scarification periods 14, 21 and 35 days exhibited normal cortex with tightly packed cortical lymphocytes and the medulla showing highly expressed lymphocytes separated with corticomedullary epithelium (Fig. 8 A, D and G).

The examined bursae of vaccinated birds (G2), at 14 days demonstrated enlarged lymphoid follicles. The lymphoid follicles showed moderate lymphoid depletion and necrosis of medulla, with presence of caseous material in the center of the lymphoid follicle (Fig. 8 B &C). On 21 days, the infolding of the corticomedullary epithelial layer with moderate lymphoid depletion and medullary necrosis were observed (Fig. 8 E &F). The histopathological changes at 35 days were characterized by slight depleted medulla associated with congested interstitial blood vessels and edema (Fig. 8 H). Hyperplasia of epithelium with epithelial cyst was also detected (Fig. 8 I).

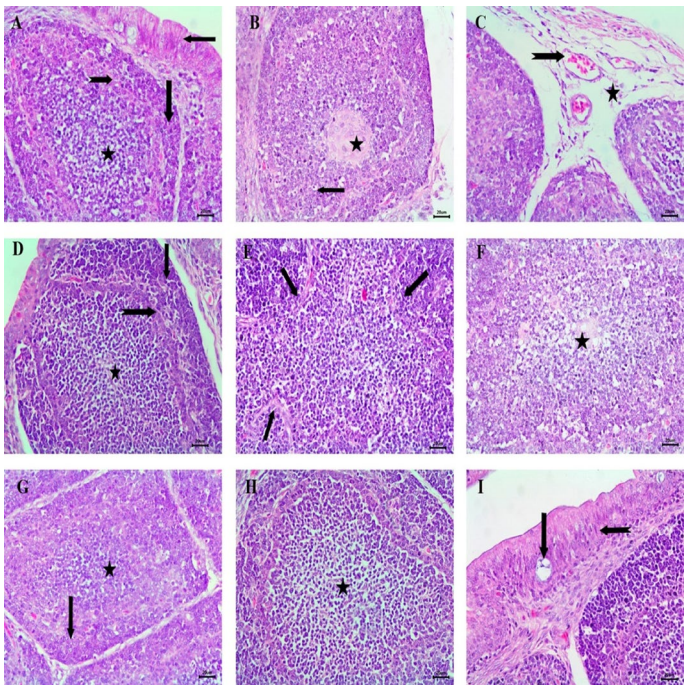

Fig. 8. Histopathological examination of bursa from experimental birds: Control group (G1) (A) at 14 days (D) at 21 days (G) at 35 days showing normal lymphoid follicles with cortical lymphocytes (down arrow) and the medulla showing highly expressed lymphocytes (star) separated with corticomedullary epithelium (notched arrow) lining with lymphoid epithelium (left arrow). Vaccinated group (G2) at 14 days (B) moderate lymphoid depletion and necrosis of medulla, with presence of caseous material in the center of the lymphoid follicle (star), (C) congested interstitial blood vessels (notched arrow), oedema in the interstitial tissue (star). At 21 days (E) infolding of the corticomedullary epithelial layer (arrows) (F) moderate lymphoid depletion with medullary necrosis (star). At 35 days (H) medulla with slight depleted lymphocytes (star) (I) hyperplasia of epithelium (left arrow), with epithelial cyst (down arrow) (bar = 20), (H&E).

The histopathological scoring of bursal lesions in different groups at 14, 21 and 35 days was demonstrated in Table 1.

Table 1. Bursa lesion score.

Groups	7 days	14 days	21 days	35 days
G1	0.00±0.00[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
G2		3.30±0.05[a]	2.60±0.11[a]	1.95±0.25[a]

Discussion

The purpose of the study was to evaluate effects of the intermediate plus IBDV vaccination that originated from tissue culture (INP-TC IBDV) (Bursipharm®). The comparison between the vaccinated and non-vaccinated groups focused on the immunological responses and histopathological effects on the bursa of Fabricius.

Both the vaccinated group (G2) and the control non-vaccinated group (G1) in this trial showed no clinical symptoms or mortalities. This result may suggest that the used INP-TC IBDV vaccine (Bursipharm®) was safe over the course of the experimental period. This agrees with Amer *et al.* (2008), who found that using live (intermediate plus) IBDV vaccines could protect the birds from the development of clinical signs and mortalities.

In the present study, there were no significant differences in body weight between the control (G1) and vaccinated (G2) groups during the experiment. This suggests that INP-TC IBDV vaccine (Bursipharm®) did not adversely affect the body weight of the broilers, this assumption agree with Al-Mayah and Tabeekh (2010) and Chansiripornchai and Sasipreeyajan, (2005) who found that there were no significant differences in body weight between vaccinated and control groups at 28 days. The feed conversion ratio (FCR) data also in this study indicated only significant increase in FCR in the vaccinated group during at 14th days. This increase could be attributed to the temporary stress induced by the vaccination process.

The obtained results revealed no significant differences in the relative weight of the bursa of Fabricius between the groups throughout the experiment, indicating that INP-TC IBDV vaccine (Bursipharm®) did not cause atrophy of the bursa of Fabricius. The BB index values further supported this, as they remained above 0.7 throughout the experiment, suggesting that there was no atrophy occurred in the vaccinated group. This aligns with the standards set by Raji *et al.* (2017) for classifying the impact of IBD vaccines. This finding does not go with the studies that investigated the effects of intermediate and intermediate-plus (IBD) vaccines on broiler chickens. Al-Mayah and Tabeekh (2010); Chansiripornchai and Sasipreeyajan (2005) and Rautenschlein *et al.* (2007) indicated that the used vaccines caused atrophy of the bursa (bursa: body weight). This discrepancy could be attributed to the fact that our used vaccine is tissue culture origin, which tends to have fewer negative effects compared to chicken embryo origin vaccines.

In the current study, the relative weight of the spleen showed no significant differences between the groups, supporting that the vaccine did not adversely affect these key immune organs.

In terms of immune response, the Newcastle Disease Virus (NDV) antibody titers measured by the hemagglutination inhibition test showed no significant differences between the groups at 21 and 35 days; however, previous studies suggested that the intermediate plus IBD vaccine inducing a significant decrease in ND antibody titers.

The IBDV antibody titers measured by ELISA showed a high significant increase ($p < 0.001$) in the vaccinated group at 35 days compared to the control group, which indicated that Bursipharm® vaccine produced protective immunity increase till 35 days (21 days post vaccination). Comparable observation also described by Meher *et al.* (2022) who used the intermediate plus vaccine (GM97 strain) at 7 days and 18 days of age and found that the intermediate plus vaccine (GM97 strain) developed the protective antibody that persists for more than 21 days. This result revealed that the INP-TC IBDV vaccine (Bursipharm®) was non-immunosuppressive and was able to induce antibody levels in chickens with maternal derived antibodies.

Histopathological examination revealed that the bursa lesion scores (Table 1) were significantly higher in the vaccinated group compared to the control group at days 14th, 21th and 35th. This suggests that the INP-TC IBDV vaccine (Bursipharm®) vaccine induced mild to moderate histopathological changes in the bursa of Fabricius. The scores indicated that while the vaccine caused some degree of lymphoid depletion and structural changes in the bursa, these changes were not severe enough to be considered pathological atrophy, as supported by the BB index results.

These histopathological findings are comparable to those reported by Hussein *et al.* (2018), where lesions were scored based on the degree of degeneration and necrosis in the bursa. Also, Meher *et al.* (2022) found that the intermediate plus strain induced significantly higher bursal

lesions at 14 and 21 days post vaccination. The mild to moderate lesions observed in this study suggest that the INP-TC IBDV vaccine (Bursipharm®) is a balanced option, providing effective immunization without causing significant damage to the bursa that usually occurs with an intermediate plus vaccine.

However, the lesion scores decreased over time, indicating tissue regeneration of the bursa. This demonstrates that the vaccine (Bursipharm®) overcame the high maternal antibody and initiated an immune response at 7 days. This matched with Hamad *et al*. (2020), who elucidated that even in the presence of high maternal derived antibodies; the intermediate-plus vaccines can reach the bursal tissues within 6–12 h post-vaccination.

Our result suggested that early immunization with the INP-TC IBDV vaccine (Bursipharm®) may lead to the occurrence of placeholder effects at bursa tissue.

The placeholder effect was facilitated by the INP-TC vaccine's ability to stimulate a strong, broad, and early immune response plays a crucial role in preventing infections by novel variant strains of IBDV. The second doses at 14 days can stimulate a strong immune response against IBDV, providing enhanced protection against the virus till 35 days.

Conclusion

Our findings support the use of this vaccine, INP-TC IBDV vaccine (Bursipharm®) in commercial broilers at an early age, especially in regions facing novel variant IBDV strains, as it can overcome maternal antibodies, initiating immune responses as early as possible, and making competitive exclusion in the bursa of Fabricius, which can prevent replication of variant strains at an early age. The second dose at 14 days can stimulate a strong immune response against other oldest strains, providing enhanced protection against the virus for 35 days without harmful effect on overall growth performance.

Conflict of interest

The authors have no conflict of interest to declare.

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