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Protective efficacy of commercial infectious bursal disease vaccines in broiler chickens following challenge: Clinical, gross pathological, and histopathological evaluation

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Abstract

The impact of the avian disease/virus (due to its acute clinical disease) on broiler production continues to be a significant viral challenge to global production and opens the door to potential industry-wide outbreak due to its association with high mortality and bursal degeneration and consequent immunosuppression. This study compared the relative protective efficacy of four commercially available infectious bursal disease vaccines for broiler chickens after a challenge from a local field isolate of IBDV. Clinical, mortality, gross pathologic, and histopathologic indicators were used to evaluate relative protection among vaccinated broiler groups.

A total of 250 Ross 308 broiler chicks were divided into five groups (50 birds/group), with four groups receiving a different type of vaccine against Infectious Bursal Disease (IBD) and one group not receiving a vaccine at all. At 16 days of age, each of the four vaccinated groups was exposed orally to a previously verified field isolate of IBDV (IBDV virus), and the groups' response to the virus was evaluated according to clinical symptoms and signs, mortality, gross anatomical changes, and histopathological changes found in the bursa of Fabricius and in the kidneys of the birds.

The vaccinated group had less severe clinical signs and lower mortality than the unvaccinated group. The unvaccinated control group experienced the highest mortality rate, 56%, while the vaccinated groups had lower mortalities via group 1 at 4%, group 2 at 12%, group 4 at 14%, and group 3 at 18%. The most severe lesions were found on both gross and microscopic examination of the bursa of Fabricius in the control group, while the vaccinated groups had various degrees of markedly reduced pathology. Group 1 had the least severe lesions and the greatest bursal and renal architecture preservation.

To conclude, all tested commercial vaccines for Infectious bursal disease virus (IBDV) elicited varying levels of post-challenge protection; Group 1 produced the most significant overall level of protection under the experimental conditions assessed. Overall, vaccination resulted in reduced clinical severity, reduced mortality, and reduced tissue lesions.

Keywords: Infectious Bursal Disease Virus (IBDV), Broiler Chickens, Vaccine Efficacy, Challenge Study, Clinical Signs, Mortality, Gross Pathology, Histopathology, Bursa of Fabricius, Kidneys, Protective Efficacy.

1. Introduction

Gumboro (Infectious Bursal Disease, IBD) is still one of the biggest viral threats to broiler chicken production associated with causing acute disease, death and immunosuppression in young chickens. The infectious bursal disease virus (IBDV) is responsible for the disease and has a specific affinity to immature B lymphocytes in the bursa of Fabricius resulting in lymphoid depletion and lack of humoral immunity (Van den Berg 2000; Dey et al. 2019).

Factors affecting the severity of IBDV infection are related to the virulence of the infectious agent (IBDV) and include some combination of age at exposure (exposure being the trigger for disease), immune system status (B- and T-cell lineage), and previous vaccination against IBDV (Van den Berg, 2000; Müller et al., 2003). In a susceptible commercial broiler-type chicken population, signs of an IBDV infection include depression, ruffled feathers, diarrhea, and variable rates of mortality, whereas the bursa of Fabricius remains the primary site of gross and histological lesions resulting from an IBDV infection (Van den Berg, 2000; Müller et al., 2003).

The primary method of controlling IBD is through vaccinating the animals. However, the various commercial vaccines that are available may provide different levels of protection from post-challenge clinical severity, mortality, and tissue damage. Therefore, a comparative post-challenge evaluation provides biologically relevant evidence of the effect of the vaccines and their protective efficacy under experimental conditions (Alkie and Rautenschlein 2016, Dey et al 2019).

The bursa of Fabricius is the main target for IBDV, and thus, the histopathological evaluation of bursal damage is a major indicator of disease severity and vaccine efficacy. Extra-bursal lesions can also occur in severe cases, which reflect greater pathological burden due to infection (Etteradossi & Saif, 2013). Experimental studies have also demonstrated that the severity of bursal damage depends on viral virulence and prior immunity; therefore, tissue-level evaluation is an important aspect of post-challenge studies (Liu et al., 2010).

The current study was performed to compare the effectiveness of three types of commercially available IBD vaccine against a field isolate of IBDV in broiler chickens. The comparison included clinical signs, mortality rates, pathological changes observed in the bursa or spleen, and histopathologic findings.

2. Materials and Methods

2.1. Experimental birds and housing

The study involved 250 Ross 308 one-day-old broiler chicks (the chicks were raised in a controlled environment under standard husbandry practices and placed into separate isolation pens as per treatment group for the duration of the experiment). The chicks had access to feed and water on an ad libitum basis. For the entire duration of the experiment, as defined by their respective treatment groups, the environmental factors affecting the chicks, such as temperature, ventilation, and lighting, were kept within the range recommended for broiler management.

2.2. Experimental strategy and vaccination practice

The birds stood randomly assigned into five equal groups, each comprising of 50 chicks. Four groups were vaccinated at one day of age with different commercial infectious bursal disease (IBD)

vaccines, whereas the fifth group was maintained as an unvaccinated control. The vaccination schedule 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
- **Group 5 (G5):** Unvaccinated control group

In addition to the experimental IBD vaccination program, all groups received the conventional routine vaccines used under local broiler production conditions.

2.3. Field isolate and challenge virus preparation

The challenge virus used in this study was prepared from a local field isolate of infectious bursal disease virus (IBDV) obtained from broiler flocks in Babylon Province showing clinical and pathological findings suggestive of Gumboro disease. The isolate had been previously confirmed as IBDV by conventional RT-PCR.

For preparation of the challenge inoculum, infected bursae of Fabricius were collected from diseased birds. Briefly, 5 g of bursal tissue was homogenized with 10 g of sterile sand in a mortar, followed by the addition of 45 mL phosphate-buffered saline (PBS; pH 7.4). The homogenate was centrifuged at 2500 rpm for 20 min, and the collected supernatant was recentrifuged under the same conditions. Penicillin (1000 IU/mL) and streptomycin (10 mg/mL) were added to the final supernatant to minimize bacterial contamination, and the inoculum was stored at -20°C until use.

2.4. Challenge test

At 16 days of age, birds in all experimental groups were challenged orally with 0.2 mL/bird of the prepared field-virus inoculum. Following challenge, the birds were observed daily for 8 days for the development of clinical signs and mortality. Clinical response was recorded comparatively among groups with particular attention to depression, ruffled feathers, anorexia, whitish watery diarrhea, reluctance to move, and rapid deterioration.

2.5. Recording of mortality

Mortality was recorded daily during the 8-day post-challenge period. Dead birds were counted for each group, and the mortality rate was calculated as the percentage of total birds in each group. Mortality design stayed used as one of the major indicators of post-challenge protective efficiency.

2.6. Gross pathological examination

Birds that died due to the challenge, together with reference birds that died from the same infection and were evaluated by necropsy, were subjected to postmortem evaluation as a part of this study. The gross pathological lesions were compared among the groups of birds evaluated by each of the treatment groups. Several parameters were evaluated with the bursal enlargement, edema and hemorrhagic changes being closely examined as well as those birds that exhibited hemorrhage(s) in the thigh muscles and other visible pathological conditions characteristic of the expression of

clinically important pathological changes associated with infectious bursal disease (IBDV) virus infection were examined.

2.7. Histopathological examination

For histopathological assessment, post-challenge tissue samples from the bursa of Fabricius and the kidney were taken from representative birds in each group according to the method outlined. These tissues were fixed in 10% buffered formalin for a period of 24 hours before being processed routinely, embedded in paraffin wax, sectioned to 5 micron thickness, and stained with hematoxylin and eosin.

Stained tissue samples were evaluated under a light microscope; representative photomicrographs were taken. Histopathological evaluation identified lesion severity and distribution throughout each organ. Within the bursa of Fabricius histopathological findings included lymphocyte depletion, follicular atrophy, apoptosis, necrosis, hemorrhage, widened inter-follicular septa and abnormal architecture. Kidney tissue samples were analyzed for interstitial hemorrhage, infiltration of mononuclear inflammatory cells, vascular congestion, and degeneration of tubular epithelial cells with cellular debris in renal tubule.

2.8. Assessment of protective efficacy

To evaluate each vaccine's level of protection from disease, all four vaccines were compared to each other based on their results after challenge. Assessment was performed based on the degree of clinical signs, deaths, necropsy findings (macroscopic lesions), and histopathology (microscopic lesions) seen in the bursa of Fabricius and the kidney. Fewer deaths, milder clinical signs, and less pathology were interpreted as providing greater protection due to the vaccine.

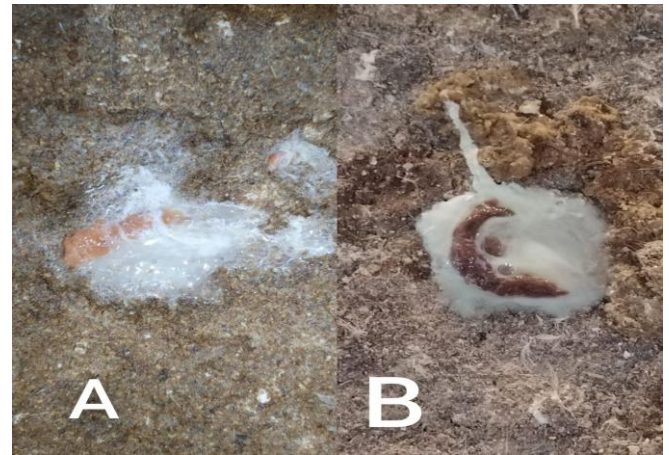
3. Results

3.1. Clinical signs after challenge

Subsequent oral contact with the field isolate of transmissible bursal sickness virus (IBDV), clear alterations in medical response stayed detected among the experimental groups. In general, the vaccinated groups showed milder post-challenge clinical manifestations than the non-vaccinated control group. In vaccinated birds, the recorded signs were mainly limited to mild depression, ruffled feathers, and reduced activity in some individuals. In contrast, the non-vaccinated control group exhibited the most severe clinical picture, characterized by marked depression, ruffled feathers, anorexia, whitish watery diarrhea, reluctance to move, and rapid deterioration in some birds, followed by death during the early post-challenge period. Overall, the clinical response indicated that vaccination reduced the severity of disease after challenge, although the degree of protection differed among the tested groups.



(Figure1) Broiler chicken from the non-vaccinated challenged control group (G5) showing depression, ruffled feathers, weakness, and a hunched posture after challenge with the field isolate of IBDV.



(Figure2). Gross pathological appearance of the bursa of Fabricius in the non-vaccinated challenged control group (G5). (A) Severe gelatinous edema around the bursa of Fabricius. (B) Enlarged edematous bursa with marked hemorrhagic discoloration, consistent with acute IBDV-associated gross lesion.

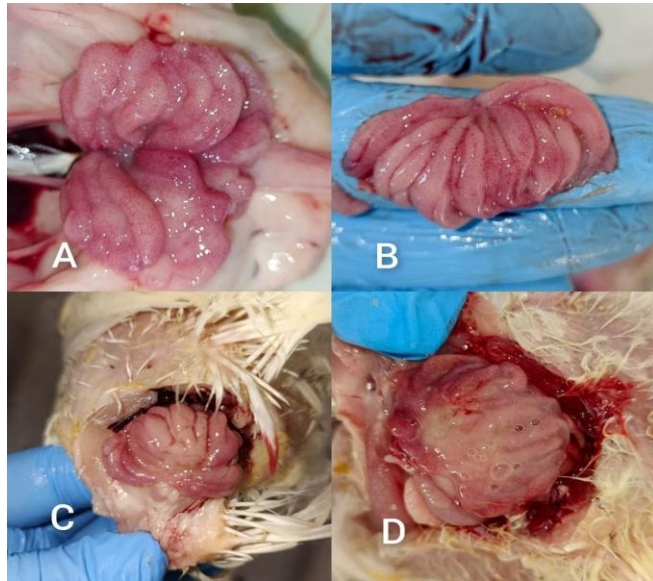
3.2. Mortality after challenge

Marked variation in mortality was observed among the experimental groups during the 8 days following challenge with the field isolate of IBDV. The non-vaccinated control group showed the highest mortality rate, with 28 out of 50 birds dead, representing 56%. In contrast, all vaccinated groups exhibited considerably lower mortality rates. The lowest mortality was recorded in Group 1 (G1), vaccinated with Vaksimune IBD MVN 002 strain, in which only 2 out of 50 birds died (4%). This was followed by Group 2 (G2), vaccinated with Vaxxon IBD, with 6 out of 50 birds dead (12%), and Group 4 (G4), vaccinated with CAVAC Transmune, with 7 out of 50 birds dead (14%). Among the vaccinated bird populations, Group 3 (G3), who received BursaPlex (though it was previously identified as non-lethal upon vaccination) had the highest group (18%) of individual birds die, with a total of nine out of fifty birds (18%). All vaccination programs significantly reduced mortality following the challenge compared with the nonvaccinated control animals; however, Group 1 provided the best protection against lethal challenge.

3.3. Gross pathological lesions after challenge

Alterations or changes at gross pathology places at once differences in the experimental groups post-mortem challenge after necropsy. Gross pathological lesions that had occurred in connection with challenge were the greatest in the group of non-vaccinated control birds, while the vaccinated groups had lesser degrees and extent of pathological changes relative to those seen in the non-vaccinated birds. The bursa of Fabricius of the non-vaccinated control birds was greatly enlarged, and there was significant edema as well; moreover, there were evident hemorrhage changes at the site of bursal hematoma. The thigh muscles of these same birds also showed signs of hemorrhaging. The lesions seen in the non-vaccinated birds; were much more severe and widespread than those seen in any of the vaccinated birds. In the vaccinated groups there were visible gross lesions, albeit of a less severe nature and extent in comparison to the non-vaccinated birds. At the bursal site, the extent of gross lesions in vaccinated birds varied; some had small to moderate degrees of

edema, some had extensive lesions at the hearts and some had none or slight lesions at the bursal sites post mortem. At the site of thigh muscle tissue; some vaccinated birds had evidence of hemorrhaging; however, the degree and extent of hemorrhaging were less than those observed in the control group. Taken together, the gross pathology of the affected birds indicated that vaccination reduces the severity of pathological changes resulting from challenge exposure.



Postmortem examination of G5 revealed marked enlargement and edema of the bursa of Fabricius with obvious congestive and hemorrhagic changes, consistent with severe gross pathological involvement following challenge.

3.4. Histopathological changes in the bursa of Fabricius

Microscopic examination of the bursa of Fabricius demonstrated clear variation in lesion severity among the experimental groups. In general, the vaccinated groups showed variable degrees of lymphoid and structural alteration, whereas the non-vaccinated control group exhibited the most severe pathological changes. In G1, the bursal tissue showed mild reactive changes in the lymphoid follicles with relative preservation of the general lymphoid architecture. Although minor structural alteration was evident, the follicular pattern remained largely maintained, indicating limited tissue injury. In G2, the bursa revealed apoptotic changes in lymphoid cells accompanied by multiple empty spaces within the follicular medulla, indicating lymphoid depletion; however, the lesion remained moderate and did not progress to extensive structural destruction. In G3, the bursal tissue showed degenerative and necrotic changes associated with hemorrhagic areas and lymphoid depletion, reflecting a more advanced degree of follicular injury than that observed in G1 and G2. In G4, the bursal section demonstrated hemorrhagic changes associated with depletion and degenerative alteration of lymphoid tissue, representing moderate to marked tissue injury, although still less severe than that observed in the control group. The most severe lesion was recorded in G5, in which the bursa of Fabricius showed severe atrophy of lymphoid follicles, marked depletion of lymphoid tissue, widening of the interfollicular septa, and obvious disruption of the normal bursal architecture.

3.5. Histopathological changes in the kidney

Microscopic examination of the kidney also showed variable pathological changes among the experimental groups. In general, renal lesions were mild in the better-protected groups and became

progressively more severe in the less-protected groups, with the non-vaccinated control group showing the most pronounced injury. In G1, the kidney showed mild mononuclear cell infiltration with mild degenerative changes in the renal tubules, indicating limited renal involvement. In G2, the renal section demonstrated degeneration of tubular epithelial cells with interstitial mononuclear cell infiltration and cellular debris within some renal tubules, indicating moderate renal injury. In G3, the kidney showed interstitial inflammatory-cell infiltration associated with vascular congestion and degenerative changes in renal tubules, reflecting a more evident degree of renal damage than that seen in G1 and G2. In G4, the renal tissue exhibited marked interstitial hemorrhagic changes associated with degenerative injury of adjacent renal tubules, indicating pronounced pathological involvement. The most severe renal lesion was observed in G5, where the kidney showed severe interstitial hemorrhage associated with marked degenerative changes in renal tubules, indicating extensive renal damage in the absence of vaccine-induced protection.

3.6. Overall post-challenge protective pattern

When the post-challenge results were measured collected, a clear gradient of defence was apparent among the investigational groups. The non-vaccinated control group consistently showed the most severe clinical disease, the highest mortality rate, the most extensive gross lesions, and the most destructive histopathological changes in both the bursa of Fabricius and kidney. In contrast, all vaccinated groups showed reduced disease severity. Among them, G1 exhibited the most favorable post-challenge profile, as reflected by the lowest mortality, milder clinical manifestations, and better preservation of both bursal and renal tissue architecture. G2 showed the next most favorable pattern, whereas G3 and G4 demonstrated intermediate protection with more evident pathological changes. Overall, these findings indicate that the tested vaccines differed in their capacity to reduce the clinical and pathological consequences of IBDV challenge, with G1 providing the strongest level of post-challenge protection under the conditions of the present study

4. Discussion

The present study showed that post-challenge protection against infectious bursal disease virus (IBDV) differed clearly among the tested commercial vaccines, as reflected by variation in clinical severity, mortality, gross lesions, and microscopic tissue damage. This pattern is biologically important because IBDV is a highly contagious and immunosuppressive virus of young chickens with marked tropism for immature B lymphocytes in the bursa of Fabricius, and therefore effective protection should ultimately be expressed as reduced disease severity and reduced tissue injury after challenge rather than by vaccination status alone (Van den Berg, 2000; Dey et al., 2019; Alkie & Rautenschlein, 2016).

A major finding of the present study was the marked contrast in clinical response between vaccinated and non-vaccinated birds following challenge. The symptoms seen in the control group are typical of a case of IBD (infectious bursal disease) in susceptible chickens. Specifically, the signs were: severe depression, ruffled feathers, anorexia (loss of appetite), whitish, watery diarrhea, reluctance to move, and rapid deterioration. These signs are commonly associated with a virulent IBDV infection and provide evidence of the rapid, systemic and lymphatic impact of the virus in an unvaccinated (non-prevented) bird. In contrast, the clinical response from the vaccinated chickens was considerably less than the response from the unvaccinated chickens, which demonstrates

that vaccination significantly reduced the clinical response from the viral challenge. The degree of protection afforded by each vaccine was different from each other (Van den Berg, 2000; Müller et al., 2003).

The mortality pattern revealed one of the best indicators of protective efficacy from the experiment conducted in this study. The marked mortality seen in the unvaccinated control group confirmed the virulence of the challenge isolate and therefore validated the biological severity of the challenge model. In contrast, all vaccinated groups experienced an overall marked reduction in mortality, indicating that vaccination had provided measurable levels of protection against lethal disease. This variation in mortality between vaccinated groups was expected given that there is biological plausibility for differences in vaccine performance against IBDV based on vaccine strain, attenuation, replication rates, and interactions with maternal antibodies (Alkie and Rautenschlein 2016; Hamad et al 2020).

The gross pathology of the lesions supports the protection level against the IBD virus and reinforces this protection level through the severity of the bursa of Fabricius, which had severe swelling, edema, and bleeding; and thigh muscle bleeding, classical lesions of acute IBD and represent the IBD virus destruction to lymphoid tissue and vascular integrity. The level of these inflammatory lesions is significantly higher than in the vaccinated groups and establishes the conclusion that unvaccinated birds were fully exposed to the pathological effects of the challenge by the IBD virus. Conversely, the severity and extent of disease lesions in the vaccinated birds were lower in the vaccinated than in the unvaccinated groups and indicate that vaccinations reduce the pathological burden associated with an IBD virus infection, although vaccinations do not always prevent lesions. This provides evidence consistent with prior research on IBDV and comparative vaccination studies that suggest that birds may develop some type of lesion after challenge even when vaccinated; however, the lesions will usually be less severe than those developed in the unvaccinated birds (Van den Berg, 2000; Dey et al., 2019; Hamad et al., 2020).

Histopathological examinations revealed that bursa of fabricius provided substantial support for the protective order observed between the groups. Severe follicular atrophy, marked lymphoid depletion, increased inter-follicular septa width, and abnormal bursal architecture in the control group represent the classic microscopic characteristics associated with advanced infection caused by IBDV. This is completely in line with the established target-finding ability of IBDV to find immature B-lymphocytes, as well as indicating that the bursa is the primary site of pathology associated with IBDV infection. Although the relative degree of preservation of the follicles within group 1 suggests the vaccine used in group 1 provided greater foundational protection against IBDV than did any other vaccine tested. Groups 2, 3 and 4 had moderate to high degrees of damage, and therefore provide evidence to suggest that while they did provide some protection against IBDV there was still substantial destruction of the bursa of fabricius in each of these groups and that the bursa of fabricius was not protected from IBDV (Alkie and Rautenschlein, 2016; Liu et al., 2010; Eterradossi and Saif, 2013).

The kidneys of all birds exhibited renal lesions; however, they were of secondary importance to the lesions in the bursa. More importantly, the renal lesions were also graded according to the severity of damage, similar to the overall protection against the

virus. Birds with the most protection exhibited mild mononuclear cell infiltration and very little tubular degeneration, while the unvaccinated group exhibited marked tubular degeneration and extensive interstitial hemorrhage in the kidneys. The results of this study provide evidence of significant extra-bursal disease in poorly protected birds and the relationship between extra-bursal lesions and destroyed bursae in the same bird is supported by others (Van den Berg, 2000; Eterradossi, & Saif, 2013).

The combination of findings from the clinical, gross pathological/histopathological, and mortality assessments reveals that the non-vaccinated controls exhibited the most extreme pathological expressions of the disease across all parameter assessed while the vaccinated animals, across all vaccine treatment groups, displayed a mitigation of the effects due to challenge. In addition, the beneficial attributes and favourable response to vaccination that were exhibited by Group 1 were even more significant considering this particular group also exhibited a robust early immune profile early on, which may have resulted in better post-challenge protection due to an early immune priming effect. While this paper mainly addressed post-challenge clinical/pathological outcomes, there is still a consistent interpretation of the findings that effective IBD vaccines should be evaluated based upon an overall integrated biological protection and not on the basis of single isolated parameters (Hamad et al. 2020; Dey et al. 2019).

Based on the results from this experiment it can be concluded that immunization provided significant reductions in clinical and pathological outcomes following challenge by IBDV. However, the level of immunological protection differed between commercial preparations of immunologically homologous vaccines. Group 1 provided the most favorable level of protection, as indicated by reduced clinical severity, the lowest mortality, and the mildest bursal and renal lesions. Therefore, post-challenge evaluation proved to be a biologically relevant and practically informative approach for comparative assessment of IBD vaccine efficacy in broiler chickens (Alkie & Rautenschlein, 2016; Müller et al., 2003).

5. Conclusion

The present study demonstrated that commercial infectious bursal disease vaccines differed clearly in their capacity to protect broiler chickens following challenge with a local field isolate of infectious bursal disease virus. Although all vaccinated groups showed some degree of protection when compared with the non-vaccinated control group, the extent of protection was not identical among the tested vaccines. Vaccination reduced the severity of clinical signs, lowered mortality, and mitigated both gross and microscopic tissue damage after challenge. In contrast, the non-vaccinated control group showed the most severe clinical disease, the highest mortality rate, and the most extensive pathological lesions, confirming the virulence of the challenge isolate and the importance of prior immunization.

Out of all evaluated vaccines, Group 1 showed to have the most supportive outcome after being challenged. The mortality rate was lowest compared to other groups, clinical signs/disease were less severe, and more of the normal histological structure was preserved in both the bursa of Fabricius and the kidneys. The other groups provided varying levels of decreased severity when compared to the non-vaccinated control group. These results suggest that the use of clinical (i.e. symptoms), mortality, pathological (i.e. body

changes), and histopathological (i.e. tissue changes) evaluations can provide a valid means for determining vaccine effectiveness. When the current study was conducted, it would appear that there was a clear difference in the effectiveness of Group 1 in terms of reducing the clinical and pathological effects of the Infectious Bursal Disease Virus (IBDV) challenge in broiler chickens.

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