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Analysis of the immune responses of broilers vaccinated with different Gumboro vaccines after receiving Ceftiofur sodium

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ABSTRACT

The study aimed to evaluate the immune response of broilers vaccinated with the Vaxxitek® HVT+IBD+ND vaccine following treatment with Ceftiofur sodium. A total of 250 broiler chickens were divided into a control group without treatment or vaccination, a Ceftiofur sodium-treated group, a vaccinated group that received Vaxxitek® HVT+IBD+ND vaccination without Ceftiofur sodium treatment and then challenged with IBDV on the 24 th day, a vaccinated and treated group that was vaccinated with Vaxxitek® HVT+IBD+ND and treated with Ceftiofur sodium and then challenged with IBDV on the 24th day, and a conventional vaccine group that received conventional live IBD vaccines on the 10th and 18th days, along with Ceftiofur sodium treatment. The group receiving both Vaxxitek® HVT+IBD+ND and Ceftiofur sodium showed no adverse effects on RBCs and hemoglobin levels while demonstrating significant improvements in lymphocyte and monocyte counts compared to other groups with minimal lymphoid atrophy and well-preserved bursal structure in this group. Overall, the findings concluded that Vaxxitek® HVT+IBD+ND vaccination combined with Ceftiofur sodium treatment leads to a better immune response in broilers, providing effective protection against IBDV and Newcastle disease while preserving bursal structure and function.

1. INTRODUCTION

In many countries, intensive poultry production systems have resulted in frequent disease outbreaks, including infectious bursal disease (IBD), also known as Gumboro disease, which affects young chickens.

This disease has been observed for the first time about 60 years ago but has been responsible for significant losses in the poultry industry ever since. A double-stranded RNA virus, IBD virus (IBDV) is found only in young chickens as serotype 1. The virus infects the bursa of Fabricius of particularly the actively dividing and differentiating lymphocytes of the B-cell lineage in immature chickens, causing immunosuppression, morbidity, and mortality. Immunosuppression increases susceptibility to infections as well as interferes with vaccination against other illnesses (Dey et al., 2019).

According to their ability to break through maternal antibodies neutralizing the vaccine virus, a variety of modified live vaccines have been developed and classified as mild, intermediate, and intermediate plus IBD vaccines (Lukert and Saif, 1991, and Teshome et al., 2015).

Immune complex vaccines, developed as an alternative, are less likely to interfere with passive immunity. The immune complex vaccine is administered intravenously or subcutaneously at one-day old, where the "intermediate plus" vaccine virus is complexed with antibodies (Van den Berg, 2004; Müller et al., 2003). Herpesvirus of turkeys + Infectious Bursal Disease (HVT+IBD) vectored vaccines are derived from the herpes virus of turkey but contain an IBDV protective gene (VP2).

Since the discovery of antibiotics, infectious pathologies have been controlled, and feed efficiency has increased (Engberg et al., 2000).

A new chemotherapeutic agent known as Ceftiofur sodium has been introduced for use in veterinary practices (Hornish and Kotarski 2002). Since some bacterial pathogens resist existing antimicrobials, continuous research is required to develop new drugs to control these diseases. A third-generation cephalosporin, Ceftiofur sodium is one of the most widely used antibiotics. Antibiotics of this class are effective against both Gram-positive and Gram-negative bacteria, including strains that produce β-lactamases. Li et al. (2011) described it as a bactericidal compound that destroys bacteria by preventing them from synthesizing their cell walls.

Shen et al. (2024) reported that adding Ceftiofur sodium to the AI vaccine had positive effects on chick growth and gut microbiota modulation. On the other hand, Buscaglia (2013) evaluated the effects of Ceftiofur sodium and gentamicin sulfate on a commercial herpesvirus of turkey vaccine for Marek's disease. Ceftiofur sodium was not shown to affect viral titer after one hour of treatment in in vivo experiments, nor did it affect the post-vaccination viremia.

Vaccination plays a crucial role in protecting poultry from infectious bursal disease (IBD). Various modified live vaccines were used, either mild, intermediate, or intermediate plus IBD vaccines, depending on their ability to break through maternal antibodies (Saif 2003). Injecting 0.08-0.2 mg of Ceftiofur sodium subcutaneously was determined to be the appropriate dosage. Injecting one milliliter (50 mg/ml) of reconstituted solution is sufficient

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to treat 250-to-625-day-old chicks (ElSayed and ElKomy, 2015). This third-generation cephalosporin has broad-spectrum antibacterial activity against antibiotic-resistant bacteria (Al-Kheraije, 2013). As well as inhibiting both humoral and cellular immune responses to vaccines, some antibiotics are immunosuppressive (Nicholls et al., 2010). As part of this study, Ceftiofur sodium was evaluated for its effect on the immune response of broiler chickens vaccinated with the newly developed Gumboro vaccine, Vaxxitek® HVT+IBD+ND, and several IBD live vaccines, as well as its safety.

2. MATERIAL AND METHODS

Statement of Ethics
The animal study received approval from the Faculty of Veterinary Medicine at Benha University, Egypt (BUFVTM 52-11-23).

Experimental chickens
Two hundred fifty-one-day-old commercial ROSS broiler chickens were acquired from Wadi Group Poultry Hatchery (El-Sadat, Menofia Governorate) and reared on a deep litter system house, fed on a balanced ration without any medication, and provided with free access to water.

Drugs
Ceftiofur sodium, produced by Pfizer, Kalamazoo, USA. A glass vial holds 4 grams of Ceftiofur sodium in the form of sodium sterile powder. The reconstituted solution achieved a concentration of 50 mg/ml by diluting 4 grams of sterile drug powder in 80 milliliters of sterile distilled water. The injected dose was 5 mg of Ceftiofur sodium per kg of body weight, as noted by Chung et al. (2007), on the day of hatching and again on the 14th day.

IBD Viruses
IBD Vaccinal Virus
HVT IBD-ND Vaccine
MERIAL SELECT, INC., Gainesville, GA 30503, USA, VAXXITEK HVT + IBD + ND® (Batch No. Rt086) with 0.2 ml s/c injection at day old for VAXXITEK HVT + IBD + ND® (Batch No. Rt086).
Viral strains of live intermediate vaccinal IBD
CEVAC® IBD L 2500D Intermediate plus.
CEVAC® IBD L contains the Winterfield 2512 strain of Infectious Bursal Disease virus in live, freeze-dried form, which was obtained from Ceva-Phylaxia Veterinary Biologicals Co. Ltd., 1107 Budapest Szállás u. 5, Hungary.
IBA-VAC ST 1000 D Intermediate Vaccine IBD strain: It contains D78 (a moderately attenuated strain of the Infectious Bursal Disease virus), obtained from FATRO S.p.A. - Laboratory Animal Products.
Challenge virus
Very virulent IBDV was obtained from the Egyptian Lab for Poultry Health (Badr City, Al-Buhaira Governorate, Egypt) with a titer of 103.5 EID50.

Experimental design
Five equal groups of two hundred and fifty-one-day-old commercial broiler chickens were randomly divided as follows:
G1: Non-IBDV vaccinated, non-Ceftiofur sodium® treated, and challenged (control).
G2: Excenel® Medicated with (5 mg. Ceftiofur sodium/Kg. Body Weight) s/c in neck fold at day old and at 14 days of age and challenged
G3: Vaxxitek® HVT+IBD+ND vaccinated at day old (0.2 ml s/c neck fold injection) and non-Excenel® medicated and challenged with vvIBDV isolate on the 24 th day of age.
G4: Vaxxitek® HVT+IBD+ND vaccinated day old (0.2 ml s/c neck fold injection), Excenel® treated s/c (5 mg. Ceftiofur sodium/Kg. Body Weight) s/c in neck fold at day old and at 14th days of age and challenged with vvIBDV isolate on the 24 th day of age (positive control).
G5: Vaccinated with conventional live IBD vaccines at the age of 10 days, with IBA-VAC ST in water and age 18 days with CEVAC® IBD L in water and medicated with (5 mg. Ceftiofur sodium/Kg. Body Weight) s/c in neck fold at day old and at 14 days of age.

Hematological analysis
Blood samples were collected from wing veins. Hematological parameters included RBCs, lymphocytes, monocytes, and hemoglobin concentrations (Hb) that were examined according to the method described by Abdulwahid and Oleiwi (2021). The total number of RBCs was determined using the Neubauer Hemocytometer (Abuoghaba, 2018), and the number of lymphocytes and monocytes was determined using Wakenell (2010) standard procedures. The Hb concentration was measured with Sahli's hemoglobinometer (Patil et al., 2013) .

Differential Leukocyte Count
The blood samples were collected in lithium-heparin tubes. A manual 200-cell differential count was performed on routinely stained blood smears, and a manual total granulocyte count was performed in a counting chamber using an eosinophil stain. The Cell-Dyn 3500 was used for differential counts with VET 2.3, a research and development version of avian-specific software. Pearson's correlation and difference plots were used to analyze the results (Lilliehöök et al., 2004) .

Histopathological Examination
Afterwards, small tissue from bursa samples was fixed in neutral buffered formalin at 10%, dehydrated with ethanol in ascending concentrations, cleared with xylene, and embedded in paraffin wax, as part of the standard procedure for histological examination. A microtome was used to cut 5-micron-thick slices of tissue paraffin sections, which were placed on glass slides and stained with hematoxylin and eosin (H&E). This method was consistent with Bancroft et al. (2013). During the histological evaluation, a light microscope (Leica DM3000) was used to carefully examine tissue architecture, inflammation, necrosis, and other pathological changes.

Histopathological Bursa Lesion Scores
The histopathological lesion scores were assessed microscopically based on the criteria established by Muskett et al. (1979). The scoring system is as follows:
• Score 0: No observable lesions.
• Score 1: 1% to 25% of follicles exhibited lymphoid depletion (less than 50% depletion per follicle), with the presence of heterophils.
• Score 2: 26% to 50% of follicles showed significant lymphoid cell depletion (more than 75% depletion per follicle), accompanied by necrosis and heterophil accumulation.
• Score 3: 51% to 75% of follicles demonstrated nearly complete depletion, characterized by necrosis and heterophil infiltration.

• Score 4: 76% to 100% of follicles were almost completely depleted, with notable necrosis, heterophil accumulation, and possible hyperplasia or cyst formation.
• Score 5: 100% of follicles exhibited near-total depletion, resulting in loss of bursal architecture and fibrosis.

Statistical Analysis

SPSS Software (version 20.0, SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data. One-way ANOVA, accompanied by Duncan's post hoc test, was employed to compare group means. The data are expressed as mean ± SEM, with a P-value < 0.05 considered statistically significant.

3. RESULTS

Considering to Hb values, G2 showed the best result overall the other groups (P<0.05) (10.09±0.24) with no significance difference among other groups. Considering to RBCs there is no significant difference (P>0.05) between the 5 groups. Considering to PCV, G4 shows the best overall results (P<0.05) comparing to other 4 groups (29.56±0.66) with significant increase in TLC (26.17) which indicates enhancing hematological and immune response comparing to other groups. Considering monocyte values, there were no significant differences between all groups (P > 0.05). On the other hand, lymphocyte values showed no significant differences between groups (2, 3, and 4) (P > 0.05); the same groups showed significant differences (P < 0.05) with groups (1 and 2). Considering TLC values, there were no significant differences between groups (1, 2, 3, and 5) (P > 0.05); the same groups showed significant differences (P < 0.05) with group (4). Concerning PCV values, there were no significant differences between all groups (P > 0.05). Results of RBCs (x106) revealed that there were no significant differences between groups (2, 3, and 4) (P > 0.05), while the same groups showed significant differences (P < 0.05) with groups (1 and 5). Results of Hb (d/dL) revealed that there were no significant differences between groups (1, 3, and 5) (P > 0.05), while the same groups showed significant differences (P < 0.05) with groups (2 and 4).

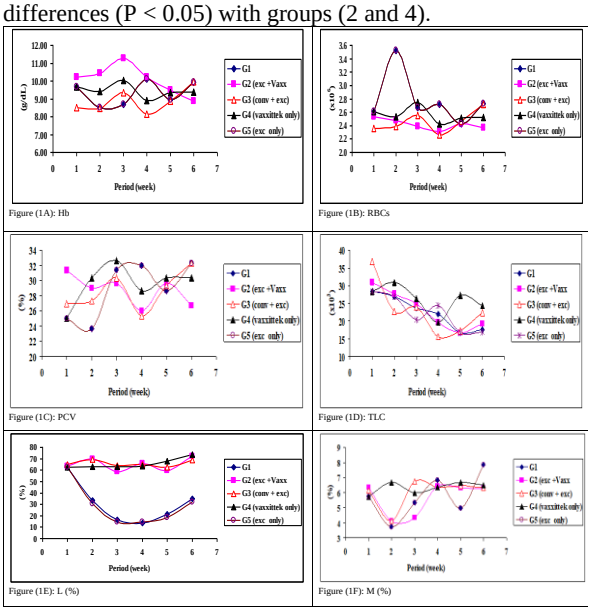

Fig (1-6). Effect of different groups on haematological profile of broiler chickens.

Histopathological results

Histopathological examination of the bursa of Fabricius tissues from the IBDV challenge group revealed marked lymphoid depletion and follicular atrophy. The germinal centers appeared less distinct, with lymphocytes replaced by eosinophilic necrotic debris. Extensive widening of the interfollicular spaces was detected (Fig. 2A), alongside severe epithelization of the follicular structures, forming multiple cysts. Some medullary cysts containing cellular debris and basophilic material were seen. Furthermore, a proliferation of interfollicular connective tissue was severe and infiltrated with mononuclear cells in most examined cases (Fig. 2B).

In the Ceftiofur sodium and IBDV challenge group, similar changes were seen in the tissues, such as the growth of interfollicular fibroplasia and several small epithelial cysts (Fig. 2C).

The conventional vaccine and IBDV challenge group exhibited hyperplasia of the follicular epithelium in some lymphoid follicles. Evidence of lymphoid depletion and follicular atrophy characteristic of an IBD challenge was also present. Interfollicular spaces showed mild widening, and infiltration of inflammatory cells was prominent (Fig. 2D).

The histopathological study of the bursal tissues from the Vaxxitek® HVT+IBD+ND, Ceftiofur sodium, and IBDV challenge groups showed that the bursa had largely returned to its normal histological structure. The lymphoid follicles of the bursa appeared hypertrophied, and there was an increase in actively proliferating lymphocytes (Fig. 2E).

The bursa in the Vaxxitek® HVT+IBD+ND and IBDV challenge group had a follicular epithelium that was still whole, and there wasn't much damage to the tissue's structure. Mild follicular atrophy and a slight widening of the interfollicular spaces were observed, but overall, the histological integrity of the bursa was well-preserved (Fig. 2F).

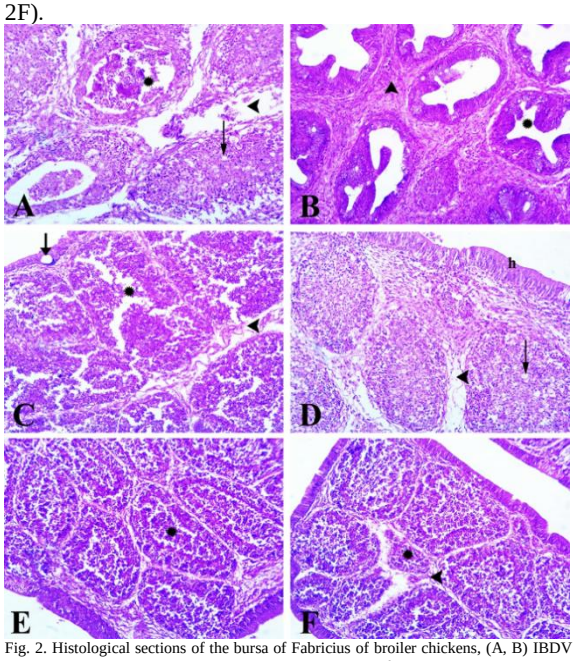

Fig. 2. Histological sections of the bursa of Fabricius of broiler chickens, (A, B) IBDV challenge group: (A) demonstrates extensive necrosis of lymphocytes, replaced by eosinophilic debris (asterisk), with significant vacuolization and lymphocytolysis in the medullary areas (arrow). Note the pronounced widening of the interfollicular spaces (arrowhead). (B) shows epithelization of the follicular structures with the formation of multiple cysts (asterisk); and there is extensive proliferation of interfollicular connective tissue infiltrated with mononuclear cells (arrowhead). (C) Ceftiofur sodium and IBDV challenge group: exhibits lymphoid depletion with atrophy (asterisk), formation of multiple small epithelial cyst (arrow), and interfollicular fibroplasia (arrowhead). (D) Conventional vaccine and IBDV challenge group: mild hyperplasia of the follicular epithelium (h), minimal vacuolization in the medullary areas (arrow), and wide interfollicular spaces (arrowhead) filled with fibrous connective tissue and mononuclear inflammatory cells. (E) Vaxxitek, Ceftiofur sodium, and IBDV challenge group: shows nearly normal histological architecture of the bursal follicles with hypertrophied lymphoid follicles and increased numbers of actively proliferating lymphocytes (asterisk). (F) Vaxxitek and IBDV challenge group: presents less severe lesions, including follicular atrophy (asterisk) and mild widening of the interfollicular spaces (arrowhead). H & E stain x200.

The IBDV Challenge group exhibits the highest average score (4.50), indicating severe lymphoid depletion, extensive necrosis, and significant interfollicular widening along with cyst formation. The Ceftiofur sodium and IBDV Challenge group, with an average score of 3.50, shows marked lymphoid depletion and partial restoration, accompanied by small epithelial cysts and ongoing fibroplasia. The Conventional Vaccine and IBDV Challenge group, averaging 2.67, demonstrates moderate follicular hyperplasia, some lymphoid depletion, and mild widening of interfollicular spaces. The Vaxxitek® HVT+IBD+ND and IBDV Challenge group has an average score of 1.83, reflecting mild lymphoid depletion with significant restoration of bursal architecture and minimal cyst formation. The Vaxxitek® HVT+IBD+ND, Ceftiofur sodium, and IBDV Challenge group had the lowest average score of 1.00, which means that there was little lymphoid atrophy and the bursal structure stayed the same, with only a small increase in the size of the spaces between the follicles.

Table 1: Histopathological bursa lesion scores for various experimental groups

Group	Scores (0-5)	Average Score	Indication
G1: IBDV Challenge	5, 5, 4, 5, 4, 4	4.50	Severe lymphoid depletion, extensive necrosis, significant interfollicular widening, and cyst formation.
G2: Ceftiofur sodium and IBDV Challenge	4, 4, 3, 4, 3, 3	3.50	Marked lymphoid depletion with some restoration, formation of small epithelial cysts, and ongoing fibroplasia.
G3: Vaxxitek and IBDV Challenge	2, 2, 1, 2, 1, 2	1.83	Mild lymphoid depletion with significant restoration of normal bursal architecture and minimal cyst formation.
G4: Vaxxitek, Ceftiofur sodium, and IBDV Challenge	1, 1, 1, 1, 1, 1	1.00	Minimal lymphoid atrophy, well-preserved bursal architecture with only slight widening of interfollicular spaces.
G5: Conventional Vaccine and IBDV Challenge	3, 3, 2, 3, 2, 3	2.67	Moderate follicular hyperplasia, some lymphoid depletion, and mild widening of interfollicular spaces.

Table 1 summarizes histological bursa lesion scores for different experimental groups, reflecting the severity of pathological changes observed following treatment and IBDV challenge. The scoring ranges from 1 to 5, with higher scores indicating more severe lesions. The average score for each group is calculated to provide an overall assessment of bursal damage.

4. DISCUSSION

Poultry producers around the world are constantly concerned about immunosuppressive diseases. The Infectious Bursal Disease Virus (IBDV) is one of the most significant immunosuppressive agents in poultry production today (Lukert and Saif, 2003). Immune suppression led to an increase in the flock's susceptibility to disease and a failure to respond to vaccination, resulting in the use of antibiotics to control the concurrent problems caused by the immune suppression. Ceftiofur sodium (Excenel®), in addition to its recommended use in poultry, also serves as an antibiotic for human use.

Taking steps to prevent early chick mortality due to colibacillosis, salmonellosis, streptococcisis, and Proteus species. The Food and Drug Administration (FDA, 1992) has approved it for the treatment of bacterial respiratory diseases in cattle, swine, and chicks.

The group that got both Vaxxitek® HVT+IBD+ND and Ceftiofur sodium did not have any negative effects on their red blood cells or hemoglobin levels. However, their lymphocyte and monocyte counts were significantly higher than those in the group that only got Ceftiofur sodium. There was also less lymphoid atrophy and more bursal structure in this group. Overall, the findings concluded that the combination of Vaxxitek® HVT+IBD+ND vaccination and Ceftiofur sodium treatment leads to a better immune response in broilers, effectively protecting against IBDV and Newcastle disease while preserving bursal structure and function.

Our results were in agreement with Ibrahim's (1999), who reported that low doses of Ceftiofur sodium significantly reduced lymphocyte transformation index in normal chickens. On the other hand, administering Ceftiofur sodium at high therapeutic dosages significantly reduced the lymphocyte transformation index in both vaccinated and non-vaccinated chickens. According to Goldstein et al. (1977), Ceftiofur sodium might induce its immunosuppressive effect by inhibiting the stem cells in the bursa and the thymus. Ceftiofur sodium induces its immunosuppressive effects by blocking bursopoeitin, a hormone that inhibits the formation of antigen-sensitive cells in the bursa and thymus. Also, Ceftiofur sodium penetrates lymphocytes and inhibits protein and DNA synthesis, suppressing cell function (Forsgren et al., 1980, and Bogert and Kroon, 1982).

In addition, Shen et al. (2024) reported that as a result of co-administration of Ceftiofur hydrochloride with AI vaccine, Escherichia-Shigella and Enterococcus abundances increased, altering membrane transport pathways, amino acid metabolism, and carbohydrate metabolism. It was found that adding Ceftiofur to the AI vaccine improved chick growth and gut microbiota modulation, although varying antibiotic concentrations and formulations could negatively affect vaccine safety and efficacy. Consequently, the inclusion of antibiotics in oil-adjuvant vaccines poses a risk of immunization failure and should be cautiously applied in poultry.

Our results agreed with Elsheikha et al. (2014), who reported that in birds medicated on Excenel, Vaxxitek® HVT+IBD+ND provides better immune response outcomes than other IBDV vaccines with the new technologies (HVT-IBD vectored vaccine); it also provides very satisfactory levels of protection against IBDV that were early and high enough to prevent chickens from being challenged by IBD viruses escaping the immunity gap between the decline in MDAs (maternal-derived antibodies) and vaccine response. In the same manner, Massi et al. (2008) reported that unvaccinated birds after challenge had severe clinical signs, and 2/15 died, while birds vaccinated with vHVT13 at day old had no clinical signs or mortality. Additionally, Le-Gros et al. (2009) reported that 4 out of fourteen Vaxxitek® HVT+IBD+ND-vaccinated birds were diseased when challenged with the vvIBD French isolate challenge virus on the 21st day post-vaccination; however, fifteen out of fifteen non-vaccinated birds were diseased.

Histopathological examination of the bursa of Fabricius tissues from the IBDV challenge group revealed marked lymphoid depletion alongside severe epithelization of the follicular structures, forming multiple cysts. The Ceftiofur sodium and IBDV challenge group showed similar histological alterations, such as the formation of multiple small epithelial cysts and interfollicular fibroplasia. The conventional vaccine and IBDV challenge group exhibited hyperplasia of the follicular epithelium in some lymphoid follicles. Vaxxitek, Ceftiofur sodium, and IBDV challenge groups demonstrated marked restoration of normal histological architecture with an increase in actively proliferating lymphocytes.

Regarding our histopathological results, Elsheikha et al. (2014) reported that examined histopathological sections from the Ceftiofur sodium-treated group showed intrafollicular infiltration with heterophiles in a few lymphoid follicles. Meanwhile, the bursa of Fabricius of chickens from groups treated with Vaxxitek®

HVT+IBD+ND with or without Ceftiofur sodium revealed an overpopulation of lymphoid follicles with lymphocytes. Our results are also in agreement with the findings of Massi et al. (2008), who found that there was almost no difference in the bursal lesion scores between vHVT13-vaccinated animals and non-vaccinated animals (control groups) when assessed on the 11th day of age before challenge. However, the results found that vHVT13-vaccinated birds were more protected than non-vaccinated challenged birds and more similar to non-vaccinated non-challenged birds on the 11 th day of age post-challenge.

5.CONCLUSIONS

From our study we can conclude that Vaxxitek® HVT+IBD+ND, Ceftiofur sodium, and IBDV Challenge group exhibited no detrimental effects on RBC and Hb levels while demonstrating a considerable enhancement in lymphocyte and monocyte counts relative to other groups. The lowest average score of 1.00 within the same group signifies minimum lymphoid atrophy and a well-preserved bursal structure, characterized by relatively modest enlargement of interfollicular gaps. Histopathological examination of the bursal tissues from the Vaxxitek® HVT+IBD+ND, Ceftiofur sodium, and IBDV challenge group revealed significant restoration of normal histological architecture. Vaxxitek® HVT+IBD+ND yields superior immune response results in birds treated with Ceftiofur sodium. It also offers excellent protection levels against IBDV and ND that were both timely and sufficiently high to safeguard the chickens against viral assaults.

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