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Effects of Live Bursal Disease Vaccine Originated From V217 Strain on Bursal Index, Morphometrical and Histological evolution of The Bursa of Fabricius in Broiler Chickens

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Abstract

Infectious bursal disease virus (IBDV) that belongs to the Avibirnavirus genus cause immunosuppressive disease in young chicken that responsible for significant economic losses in broiler chickens worldwide; therefor Hygienic management (biosecurity) and proper immunization (passive and active) are very important for the control of the disease, but live attenuated vaccines used for this purpose can cause immunosuppression because of bursal damage. Our study was to investigate about the effects of commercial IBD vaccine: V217 strain (intermediate plus/hot, administered in drinking water) on the bursa of Fabricius (BF) under broiler chickens rearing conditions. For this purpose, a total of two hundred-day old coob 500 broiler chicks have been divided into 3groups, the first group was not vaccinated (CG), the second group (G1) was vaccinated with intermediate and intermediate plus vaccine and the third group (G2) was vaccinated only with intermediate plus vaccine. Blood samples were collected to preset the IBD vaccination schedule. To ovoid stress, which might affect bursa, standard vaccines were canceled.

Ten samples from each group were randomly obtained at 25, 35 and 45 days. The following parameters from each group were studied: diameter, bursa weight, body weight, and damaged bursa in different states in order to determine the immunological status of the broiler chickens vaccinated.

Our results have revealed that the immune status of the broilers was below standard with low bursal index (BI) in the three groups. However, the morphohistologic characteristics confirmed the impact of IBD live vaccine on the bursa of Fabricius in the two groups (G1, G2); Atrophy of follicles, epithelial degeneration, fibrosis, mild to severe lymphocytic depletion and cyst formation. Nevertheless, these lesions were more accentuated in G2 but a physiological compensatory hyperplasia and lymphocytes repopulation in group G1 were mostly observed.

Key worlds: bursa of Fabricius, bursal index, histopathology, broiler chickens.

INTRODUCTION

Morphometry and histopathology of the bursa of Fabricius are two easy means, which allow a diagnosis of avian pathology to be completed in the field, especially in the event of attack caused by a vaccine or wild virus [1]. Infectious bursal disease virus (IBDV) is a non-enveloped virus with an icosahedral symmetry belonging to the Birnaviridae family [2]. Its genome consists of double-stranded RNA (dsRNA) divided into two distinct segments: Segment A: This segment is larger and encodes for the viral polyprotein (VP2-VP4-VP3) and VP5. The polyprotein is processed into individual proteins by the viral protease (VP4), VP1: necessary for viral replication, VP2: The major capsid protein, responsible for antigenic properties and host immune response, VP3: A multifunctional protein involved in viral replication and particle assembly, VP4: A protease crucial for processing the polyprotein, VP5: Plays a role in virulence and virus release; Segment B: Encodes the RNA-dependent RNA polymerase. [3].

Infectious bursal disease virus (IBDV) causes an immunosuppressive disease in young chickens; the bursa of Fabricius (BF) is the primary target organ of IBDV, resulting in a weakened humoral immune response [1, 2, 3]. The disease manifests in three clinical forms: very virulent, classical virulent, and sub-clinical. The very virulent and classical virulent forms exhibit distinct clinical signs, while the subclinical form lacks specific symptoms. Although subclinical infection does not result in mortality, it can cause significant issues such as weight loss in the flock, immune suppression, and ineffective vaccination against other diseases [8].

IBDV is very stable and resistant to many disinfectants and environmental factors [5, 6], and therefore vaccination is the most effective way of providing protection against this dangerous virus [2, 3], Nonetheless, live attenuated vaccines designed for this purpose may cause moderate to severe damage to the bursa, inducing corresponding degrees of immunosuppression, which compromising immune response to vaccination against other pathogens responsible for various types of infections, increasing susceptibility to diseases [2, 5, 4].The safety and effectiveness of this type of vaccine continue to be significant concerns [4], on the other hand estimating the optimal time of vaccination is very important by measuring the level of maternally derived antibodies (MDA) at a very young age [11].

This study aims to investigate the effects of commercial IBD vaccine on Bursa of Fabricius (BF) under routine broiler rearing conditions.

MATERIALS AND METHODS

1. Experimental Chickens and trial design

A total of two hundred-day old Coob 500 broiler chicks were obtained from the same broiler breeder flock and hatchery, have been divided into three groups, the first group was not vaccinated (Control group), the second group was vaccinated with intermediate and intermediate plus vaccine (group1) and the third group was vaccinated only with intermediate plus vaccine (group2). To avoid stress which might affect bursa, standard vaccines were canceled.

The birds were reared under the same rearing conditions in the area of Chelghoum laid in Algeria on litter floor and fed ad libitum on commercial poultry feed.

Ten samples from each group were obtained at 25,35 and 45 days of age to perform morpho-histopathological and bursal index evaluation (fig1).

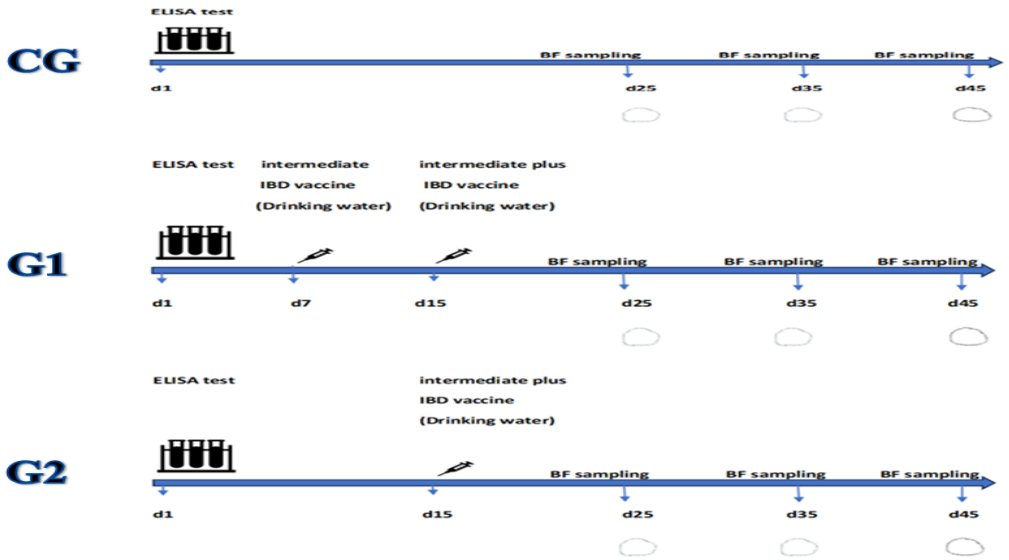

Fig1. trial design

2. Serology

Blood samples were collected from each group at the first day of age and antibodies against IBVD were measured using a commercial ELISA kit (IDEXX) (Fig2);in order to avoid the interference of the vaccine with maternally derived antibodies (MDA) [6].

Fig2. Elisa kit

3. Vaccine and vaccination

The first group was not vaccinated (**CG**).

The second group (**G1**) was vaccinated with a live commercial vaccine (intermediate LC 75 strain) at the 7th day and a live commercial vaccine (intermediate plus, 217 strain) at the 15th day of age in drinking water.

A live commercial vaccine (intermediate plus, 217 strain) was given in drinking water at the 15th day of age to the third group (**G2**).

4. Morphometric study

A special ruler that contains eight orifices with different diameters (bursometer) (Fig.3) was used to measure bursa size of birds after being sacrificed, and given scores from 1 to 8, based on the following parameters:
score 1 (diameter up to 3.17 mm); score 2 (from 3.18 to 6.35 mm); score 3 (from 6.36 to 9.52 mm); score 4 (from 9.53 to 12.7 mm); score 5 (from 12.8 to 15.87 mm); score 6 (from 15.88 to 19.05 mm); score 7 (from 19.06 to 22.22 mm); and score 8 (from 22.23 to 25.4 mm) [7].

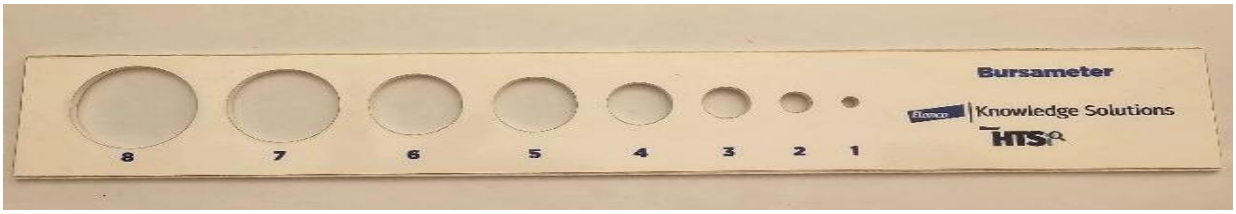

Fig3. Bursameter (Solvay animal health, 1992).

5. Calculation of Bursal Index

At 25,35 and 45 days of age, ten chicks were randomly collected and weighed to determine their body weights; the bursa of Fabricius of the euthanized chicks were removed and weighed (Fig4).

The calculation of Bursal Index (BI) was obtained according to the formulas of Lucio and Hitchner (1979): weight of BF /body weight x 100 [8].

Fig4. Determination of bursal weight and chicken's body weight.

6. Histology

After the bursas were measured and weighed, they were fixed in a buffer (10% formalin solution) for 24 hours then underwent a dehydration in ascending grades of alcohols, after that cleared in xylene and embedded in paraffin; paraffin blocks were cut to 5 µm thick sections with a microtome, The resulting sections were stained with haematoxylin-eosin (HE) [10,11].

Microscopic examination of histology slides was performed with a light microscope under low (×10) and high (×40) magnification. To make findings clearer and more accessible, photographs of certain specimens were taken for better illustration of the results.

7. Statistical analysis

Obtained data were analyzed using One-way ANOVA (analysis of variance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator for comparing among the groups.

RESULTS

The data on the development of bursa of Fabricius diameter for the three groups (Control Group (CG), Group 1 (G1), and Group 2 (G2) is presented in (Fig 5); At D 25 the size of the bursa was higher in vaccinated groups ($p < 0.05$). At D 35 the measurement of the bursal diameter in the three groups was not significantly different ($p > 0.05$) however at D 45 the size in the control group was significantly higher than vaccinated groups ($P<0.05$).With $p>0.05$ the difference in size between G1 and G2 was not significant during the experiment period.

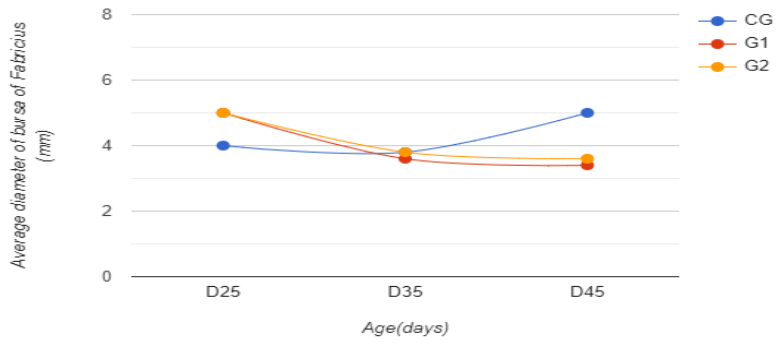

Fig5. Post-vaccination evolution of the average diameter of the bursa of Fabricius in the three groups.

The bursal index (BI) calculated during the study period mention that 100% of broiler chickens in all groups belonged to the bad class (<0.15) which illustrate the immunodepression in studied herds (table).

Groups / Class	BI> de 0.20% excellent	0.18%<IB<0.20% Average	0.15%<IB< 0.18% Medium	BI< de 0.15% bad
CG	-	-	-	100%
G1	-	-	-	100%
G2	-	-	-	100%

Table. Bursal index

The histopathologic lesions of the bursa of Fabricius taken from vaccinated groups were characterized by lymphocytic depletion, interfollicular and intrafollicular edema, intraepithelial cysts , Intrafollicular cystic cavities and corrugated epithelium nevertheless the

difference in size between G1 and G2 was not significant. These lesions were more accentuated in G2 but a physiological compensatory hyperplasia and lymphocyte repopulation in group G1 were mostly observed. (fig7, fig8, fig9).

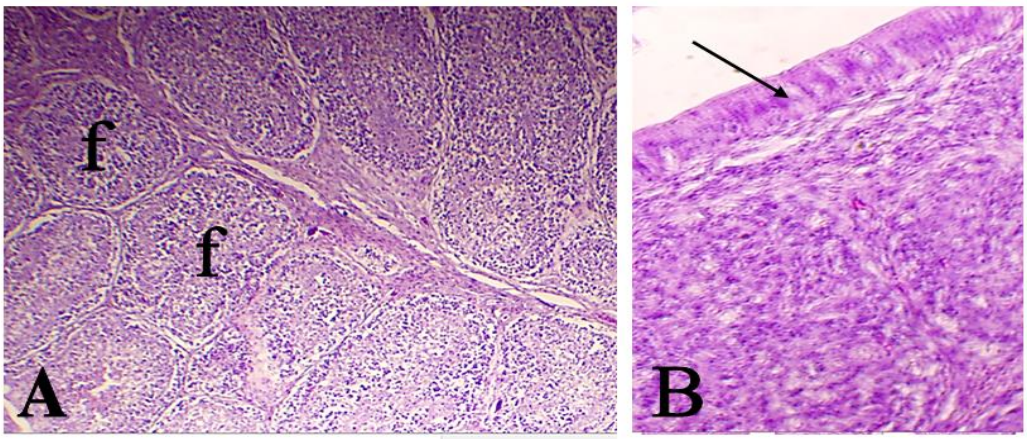

Fig 6. Normal microscopic appearance of the bursa of broiler chickens in the control group at 25 days of post aching age: A.The bursal follicles are tightly packed, with a normal concentration of lymphocytes (H&E, X100). B. Follicles are covered by pseudostratified columnar epithelium (arrow) (H&E, X400).

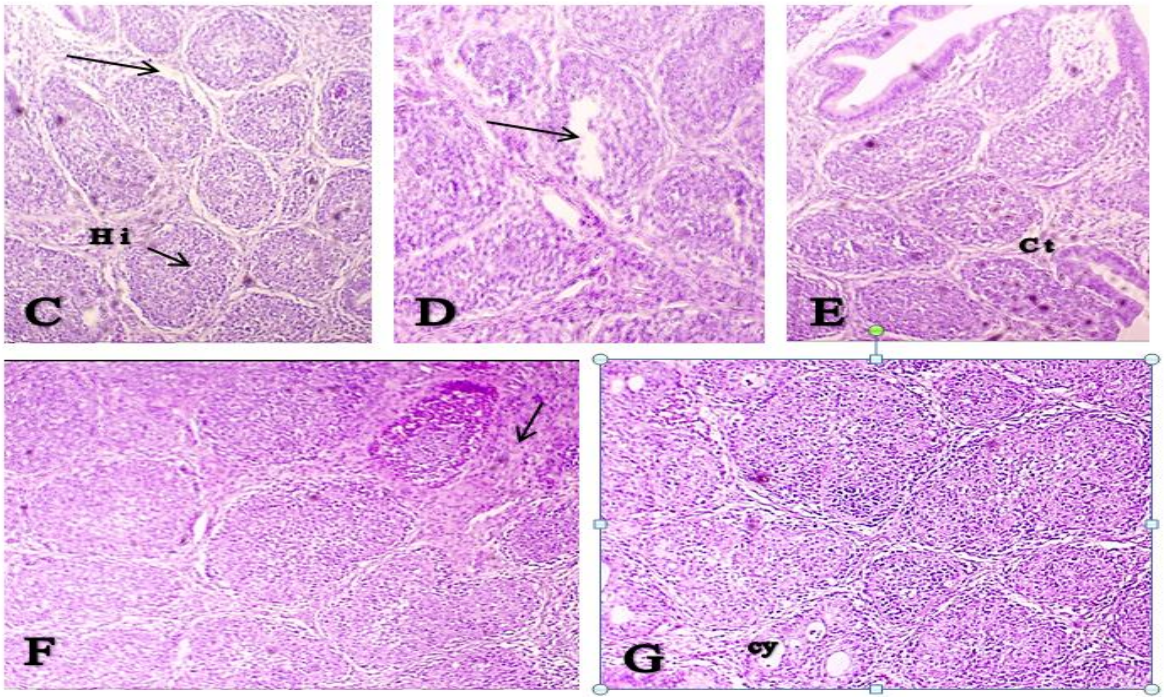

Fig7. Histopathological lesions of the bursa of Fabricius in broiler chickens of vaccinated groups at 25 days.

C. Bursal section from birds of G1 at 25 days of age showing interfollicular edema (arrow), heterophile infiltration (Hi). (H&E, X100) . **D:** Bursal section of G1 at 25 days of age showing intrafollicular edema (arrow) (H&E, X400). **E:** Increase in the thickness of interfollicular connective tissue (Ct) in the bursal section of G1 at 25 days of post aching age (H&E, X400).

F: Bursal section of G2 at 25 days of age presentation intrafollicular and interstitial heterophile infiltration (arrow) (H&E, X400). **G:** Multiple intrafollicular cysts (cy) observed in the bursal section of G2 at 25 days of age (H&E, X400).

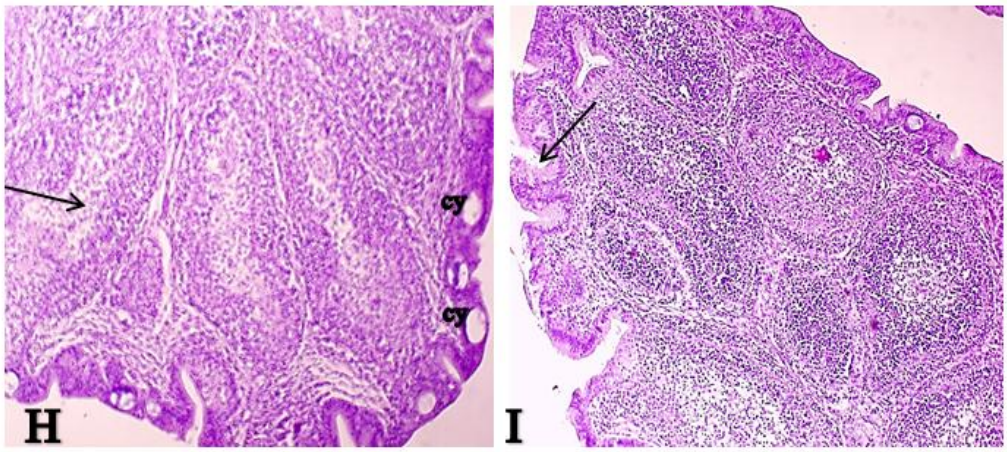

Fig 8. Bursal histopathological lesions of vaccinated groups at 35 days.
H: Section of the bursa of Fabricius of G1 at 35 days of age showing multiple intraepithelial cysts with mild lymphocytic depletion (H&E, X400). **I :** Bursal section of broilers of G2 at 35 days of age mentioned a moderate lymphocytic depletion with corrugated epithelium (H&E, X400).

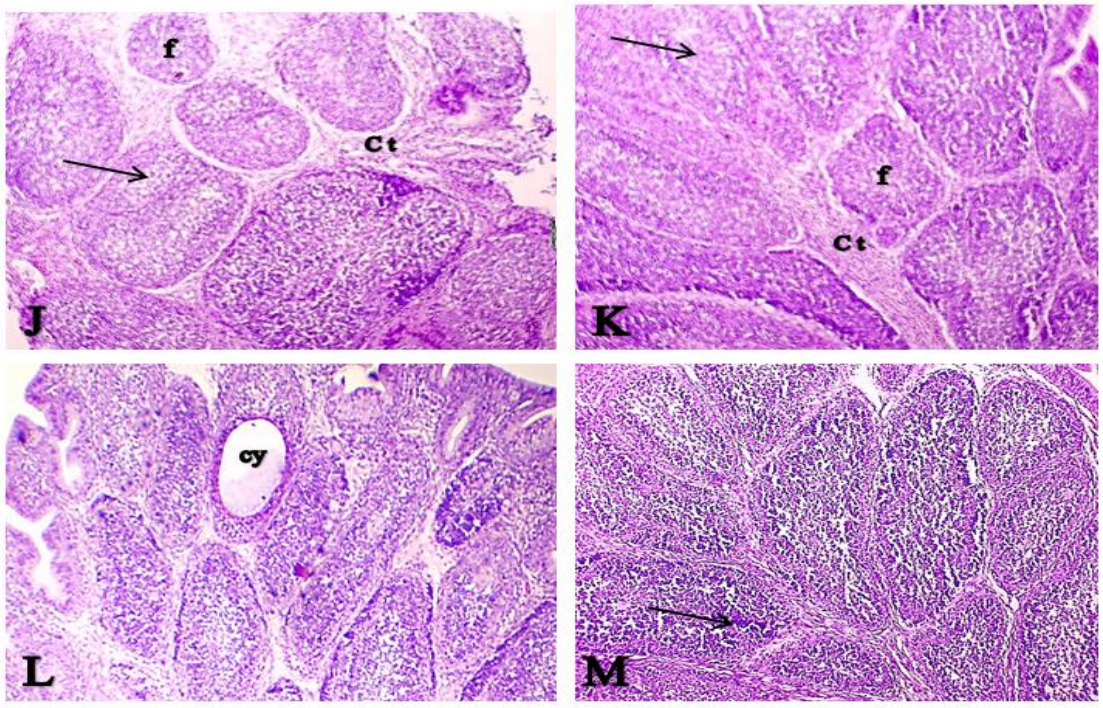

Fig 9. Histopathological lesions of the cloacal bursa of broilers in vaccinated groups at 45 days.
J-K: High increase in the thickness of interfollicular connective tissue with lymphocytic depletion and lymphoid follicles atrophy of Bursal section of G1 and G2 at 45 days of age (H&E, X100)
L: Bursal section of G2 at 45 days of age mentioned intrafollicular cyst and thickness of interfollicular connective tissue with lymphocytic depletion (H&E, X400). **M:** Bursal section of G1 at 45 days of age showing histological regeneration and B cells repopulation (H&E, X400).

DISCUSSION

The Bursal Index (BI) and morphohistologic characteristics of lesions are the key parameters for assessing the immunosuppression induced by IBD vaccination [1,12]. Narang *et al* and Amelia *et al* have reported that vaccines induced bursal damage after vaccination [12, 13] which aligns with our results. Morphometric changes (hypertrophy and atrophy) of the bursa were accompanied by different degrees of bursal tissue damage (edema, leucocytes infiltration, lymphocytic depletion, follicles atrophy) in vaccinated groups as khenenou *et al* have reported [11].

Bursal index reflects the relative changes in weight of the bursa of Fabricius with respect to body weight, according to Zahedi *et al* the bursa diameter is not proportional to the age and the weight of the bird. Sellaoui *et al* also highlighted the discrepancy between the bursal weight and the age and the body weight of the birds in their study [14, 15] which is in agreement with the results obtained. Infectious bursal disease virus is the most important agent causing immunosuppression in chickens, nonetheless there are numerous other factors, including infectious (reovirus, bacterial infections) and non-infectious (mycotoxins, stress), that can produce similar effects [13,1].

Our study has shown that the histopathological lesions correlate with higher bursa size in vaccinated groups at 25 days of age compared to the control group; this hypertrophy is illustrated by interfollicular edema and heterophile infiltration.

The evolutionary kinetics in the normal cloacal bursa at 35 days of age (control group) continues its normal development but the bursa in vaccinated groups began to decrease in size because of the lymphocytic depletion and follicles atrophy , at 45 days of age bursa in control group continues to increase in size while in vaccinated groups the bursa continues to decrease in size ; illustrated by follicles atrophy induced by lymphocytic necrosis (intrafollicular cysts) and interfollicular fibrosis .

The histological study has shown also a bursal regeneration and B cells repopulation in G1 at 45 days of age, which can positively impact the immune system.

The atrophy of the bursa of Fabricius is closely related to the intensity of histopathological lesions (lymphocytes necrosis and follicles depletion) which is consistent with results obtained by *Sellaoui et al* [15].

The reversibility of histological lesions in the bursa largely depends on whether the reticulohistiocytic system can restore its function effectively. If macrophages and other immune cells are able to clear debris, resolve inflammation, and repair the damaged tissue, healing can be more complete [11]. This organ plays a crucial role in the immune system of birds, particularly in the production and differentiation of B lymphocytes, which are essential for the adaptive immune response [2], a smaller-than-normal bursa of Fabricius may suggest developmental issues or immune dysfunction, as the organ's size is typically correlated with the strength of the immune system. A reduction in size can be a sign of immune system suppression or inadequate immune response [16].

CONCLUSION

The abnormal BI in vaccinated groups and control group is a clear sign that the immune status of the broilers is below standard. The histopathological aspects showed fibrosis, epithelial degeneration, mild to severe lymphocytic depletion and cyst formation, which confirms the adverse effect of the live vaccine on the BF. Vaccine viruses are designed to mimic the disease-causing pathogen in a controlled manner; vaccine virus replicate in immature B lymphocytes which causes degeneration, minor necrosis and inflammatory cell infiltration in the BF, however regenerative changes can be seen in the bursal follicles over time which was observed in G1 at 45 days of age, thus, giving an intermediate vaccine before giving an intermediate plus one contribute to minimize bursal damage. In the field, very virulent strains of Infectious Bursal Disease Virus (IBDV) are responsible for high mortality and significant economic losses, primarily due to their ability to continuously evolve in terms of antigenicity and virulence. These strains lead to prolonged and severe immune system suppression.

Less attenuated intermediate-plus or hot vaccines, are required to get rid of IBD outbreaks in endemic area .Therefore many factors must be estimated when selecting an effective vaccine ; Bursal damage , time required for physiological compensatory hyperplasia, and the length of the rearing period (broilers, breeders or layer hen…). The previously cited factors are crucial to obtain an effective IBD vaccination strategy.

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