



Original Article

Comparison of Pathogenicity of Four Commercial IBD Intermediate Live Vaccines in Broilers

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Abstract

This study was designed to investigate four commercial intermediate live vaccines against infectious bursal disease (IBD). One hundred and fifty, 1-day-old ROSS broiler chicks divided randomly into 5 groups. Each group was consisted of 30 birds. The birds were vaccinated with an intermediate IBD vaccines namely A, B, C, and D vaccines at 14 days of age *via* intracrop route except group 5 which was acted as control unvaccinated group. Following vaccinations, different parameters were used in this investigation including; clinical signs and gross lesions, lymphoid organs indices and histopathological changes. The result indicated that no clinical signs and gross lesions were observed on vaccinated birds. Significant increase ($P < 0.05$) in bursal index at 17th day of age in group 4, whereas a significant reduction ($P < 0.05$) at 28th days of age has been noticed which indicated bursal atrophy as compared with control and other vaccinated groups. Spleen index revealed significant reduction ($P < 0.05$) at 28th days of age in the same group as compared with control and other vaccinated groups throughout the experiment. Thymus index revealed significant reduction ($P < 0.05$) in group 1 at 28th days of age as compared with control and other vaccinated groups. Histological examination of bursa of Fabricius (BF), spleen and thymus revealed that all type of vaccines induced different degree of alterations in these organs. The organs in group 1, 2 and 4 showed similar degree of changes which characterized by an edema and degeneration in the medullary area of bursal follicles. Spleen of groups 1 and 2 showed follicular necrosis and sinusoidal congestion, whereas that of group 4 showed hydropic degeneration in the epithelial layer. Thymus in group 1, 2, 4 exhibited congestion and hemorrhage in the medulla with lymphocytic depletion. Bursa of group 3 showed thickened capsule whereas spleen showed hydropic degeneration in the epithelial layer of the blood vessels, whereas the thymus changes represented by focal area of hemorrhage. Study of the pathogenicity of four commercial IBD vaccines showed considerable variation in their pathogenicity. In conclusion, vaccine D proved to be more pathogenic than A, B, and C vaccines. This was supported by bursal, spleen and thymus reduction and bursal score indices.

Keywords: BF; Thymus index; IBD Intermediate; lymphocytic depletion.

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Introduction

Poultry industry has expanded rapidly over the last four decades and is playing a vital role in the economy of the country. However the industry is confronted with a variety of problems, particularly the diseases of viral origin. The major problem of this business is the outbreak of infectious diseases, including Infectious Bursal Disease (IBD) (Phatak, 2002).

The IBD is particularly important due to high mortalities, lowered productivity among infected chicks and immunodepression to others (Sahar *et al.*, 2004). The IBD virus replicates extensively in IgM (+) cells of the bursa and chickens may die during the acute phase of the disease, although IBD virus-induced mortality is highly variable and depends, among other factors, upon the virulence of the virus strain (Balamurugan and Kataria, 2006). The virus is resistant to a large variety of disinfectants and is environmentally very stable but may be controlled using a proper vaccination schedule. Vaccination represents a very useful method in IBDV controlling (Dacic, *et al.*, 2008).

Although live vaccines have been shown to be very efficacious against a variety of poultry diseases, two consequences of vaccinating concurrently with several vaccines are immunosuppression and vaccine interference. Immunosuppression has been associated with the use of live IBD virus vaccine as this virus replicates in cells associated with immune responses. In addition, vaccines that have similar tropisms are known to interfere with the immune response to each other (Cook *et al.*, 2001; Ganapathy *et al.*, 2005). Rautenschlein *et al.*, (2003) compared immunopathogenesis of mild, intermediate and virulent strains of classic IBD viruses and showed that the most virulent strain induced the most prominent bursal damage and significant suppression of the mitogenic response and the mild vaccines induced no detectable lesions in the bursa.

At the present time, there is more than a company which produces of the IBDV vaccines but there are more than strains differ in the virulence so the strains varying in the effect on the lymphatic system. The veterinary vaccines must be controlled and assessed by neutral and independent researcher in different countries, before admit for commercial use (Martinez *et al.*, 2002). This study was designed to determine the pathogenicity of four commercially available IBD live intermediate vaccines administered at 14-day-old chicks.

Materials and Methods

One hundred and fifty, 1-day-old ROSS broiler chicks were allotted into 5 groups, 4 groups were vaccinated with commercial freeze –dried live vaccines, namely (A,B,C and D vaccines respectively) at 14 days of age *via* intercrop route and the other one was served as control. The commercial vaccines names were known, but were not revealed to avoid commercialization.

The birds were placed into separate sterile cages at the experimental house of the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basra University under strict hygienic and standard management conditions. The vaccines were reconstituted in distilled water to obtain one field dose in 0.5 ml, and given intercrop by a blunted syringe to ensure that all birds has been received the dose of the vaccine.

At 17th and 28th day of age, five birds were randomly selected from each group and individually weighted, sacrificed and necropsied. The BF, spleen and thymus were removed and weighed before fixation for histopathological observations. Bursa, spleen and thymus/body weight ratios were calculated for each bird and expressed as arithmetic means in each group of birds by the following formula: Organ index = organ weight in grams / body weight in grams x1000 (Hedayati *et al.*, 2005).

Samples of bursa, spleen and thymus were fixed in 10% buffered formalin for histopathological examination and stained with Haematoxylin and Eosin (HE).The bursa, thymus and spleen were examined using optic microscope for histological study (Luna, 1968).

Effects of used IBD vaccines were assessed in terms of bursal, thymus and spleen indices and microscopic examination of lymphoid organs.

Bursas were scored according to Rosales *et al.* (1989) and assigned as; 1= no lesion(normal) ; 2 = focal mild lymphocytic depletion ; 3 = multifocal, 1/3 to 1/2 of the follicles show lymphocytes depletion ; and 4 = diffuse lymphocytes depletion of all follicles

The data of organ indices were subjected to analysis and the significant differences at ($p<0.05$) which were determined by two ways ANOVA Statistical software sigma stat statistical (Version 19.0, SPSS Inc, Chicago, Illinois, USA, 2010).

Results

Chicks of all vaccinated groups did not exhibit any clinical signs and there was no mortality throughout the experiment.

Bursal index (BI) was increased at 17th day and reduced at 28th day post-vaccination with live Gumboro disease vaccines in all vaccinated groups. Group 4 revealed a significant increase ($P<0.05$) among other groups which was (2.222 ± 0.131) at 17th day .On the other hand this group showed significant reduction ($P<0.05$) which was (1.400 ± 0.089) at 28th days .Significant reduction of spleen index ($P<0.05$) was also noted in G 4 (0.898 ± 0.057) at 28th day of age as compared with control group and other vaccinated groups (Table 1).

The bursal lesions scoring for the G1,G2 and G4 at 17th days post vaccination was 3 lesions, which included an edematous area in fibrous capsule and increasing of interfollicular space due to decrease number of lymphocytes and follicular atrophy(Figure.1). At 28th days the scores of G1,G2 and G4 were 4 lesions, which appeared more severe as increasing of interfollicle space, degeneration in the medullary area and depletion of lymphocytes in bursal follicles, disappearance of demarcation between cortical and medullary area and bursal follicles atrophy(Figure.2,4)

The bursal lesion score of G 3 was 2 lesions at 17th day which appeared as hyperplasia of interfollicular septa, mild depletion of lymphocytes in some bursal follicles (Figure.3). At 28th day was become 3 when characteristic feature of fibroplasia which was responsible for interfollicular connective tissue formation and thickening capsule has been occurred.

Experimental Groups and vaccines	Mean \pm SE of bursal index		Mean \pm SE of Spleen index		Mean \pm SE of Thymus index	
	17 th days	28 th days	17 th days	28 th days	17 th days	28 th days
G1(A)	*1.962 $\pm 0.013a$ A	1.692 $\pm 0.088a$ A	1.106 $\pm 0.027a$ A	0.996 $\pm 0.011a$ A	3.300 $\pm 0.200a$ A	2.740 $\pm 0.097b$ A
G2(B)	2.064 $\pm 0.097a$ A	1.674 $\pm 0.091a$ A	1.100 $\pm 0.026a$ A	0.988 $\pm 0.072a$ A	3.208 $\pm 0.210a$ A	2.964 $\pm 0.285a$ A
G3(C)	1.944 $\pm 0.034a$ A	1.702 $\pm 0.091a$ A	1.012 $\pm 0.096a$ A	1.016 $\pm 0.077a$ A	3.020 $\pm 0.148a$ A	2.928 $\pm 0.218a$ A
G4(D)	2.222 $\pm 0.131b$ A	1.400 $\pm 0.089b$ B	1.046 $\pm 0.056a$ A	0.898 $\pm 0.057b$ A	3.080 $\pm 0.131a$ A	2.862 $\pm 0.301a$ A
Control G5 Unvaccinated	1.870 $\pm 0.021a$ A	1.974 $\pm 0.017a$ A	1.144 $\pm 0.085a$ A	1.140 $\pm 0.037a$ A	3.364 $\pm 0.133a$ A	3.444 $\pm 0.218a$ A

Values followed by different letters (Capital letters= horizontally; small letters= vertically) were significantly different ($p < 0.05$) in comparison with the control group.

* Five birds in each group.

Table (1): Results of bursal, spleen and thymus indices following vaccination with commercial live intermediate IBD vaccines at different ages.

Age (days)	Experimental groups				
	G1	G2	G3	G4	G5 Control
17 th days	3	3	2	3	0
28 th days	4	4	3	4	0

Table (2): Bursal lesion scoring in experimental groups at different ages

Control unvaccinated group (G5) in the experiment was showed no alterations in the BF at 17th and 28th day of chicken age (Figure.5)

The histopathological changes of spleen of group1 at 17th day were showed follicular necrosis and lymphocytic depletion in the center of the organ (Figure.6) and mild lymphocytic depletion at 28th day.

Group 2 was exhibited follicular necrosis, depletion of lymphocytes, and sinusoidal congestion along the observation period (Figure.7).

Group 3, was showed severe thickening of the wall of the blood vessels and hydropic degeneration in the epithelial layer of the blood vessels of spleen at 17th day of vaccination with intermediate vaccine. At 28th day, the splenic lesions were seen as sinusoidal congestion with follicular atrophy (Figure.8).

The histopathological changes of G4 at 17th day were showed thickening of the wall of the blood vessels and hydropic degeneration in the epithelial layer (Figure.9) and slight follicular atrophy due to depletion of lymphocytes was present at 28th day.

The control group (G5) did not reveal any alterations during the experimental period in the spleen at 17th and 28th days (Figure.10).

The effect of vaccine on thymic tissue of G 1 was showed congestion in the medulla, lymphocytic depletion and multifocal areas of hemorrhages in the medullary area at 17th day (Figure.11), whereas mild congestion in the thymic tissue has been observed at the 28th day.

The histopathological examination of thymus of group2 at 17th day was exhibited congestion of blood vessels and severe depletion of lymphocytes in the medulla with diffuse hemorrhage. On the other hand, at the 28th day of age, vaculation and depletion of lymphocytes in medulla with diffuse hemorrhage were noticed (Figure.12). Focal areas of hemorrhages in medulla with severe depletion of lymphocytes (Figure.13) were seen in the thymic tissue of group 3 at 17th day. At 28th day of age mild depletion of lymphocytes in medulla was recorded. The tissue of thymus gland of G 4 at 17th day was displayed slight depletion of lymphocytes in medulla and a few number of multifocal areas of hemorrhages. Thymic atrophy and depletion of thymic lymphocytes (Figure.14) was showed at 28th day. The control group was appeared as normal thymic section at 17th and 28th day of age (Figure.15).

The results of the histopathological examination of the bursa, spleen and thymus gland in the vaccinated birds of present study indicated that live IBDV vaccines were enough to cause some pathological lesions in the lymphoid organ.

Discussion

Some vaccines are capable of inducing similar or more severe bursal lesions than those caused by field virus strains (Luengo *et al.*, 2001), and most of the IBDV vaccines showed variable degree of pathogenicity and immunosuppressive effect (Hussain *et al.*, 2001), as well as histopathological changes induced by vaccine virus in the various lymphoid organs are very different, depending on the virulence of vaccine strain (Rus *et al.*, 2010).

In the present study, disappearance of clinical signs and mortality might be an indication of safety of the used live IBD virus vaccines and early age resistance (Hassan, 1986; AL-Mayah, 2009).

The increasing BI after 3 days of vaccination with intermediated vaccine may be due to the acute inflammation and presence of edema with hyperemia in the bursal tissue, whereas the significant reduction of BI which was recorded in vaccine D in the present study might be explained depending on Nishizawa *et al.*, (2007) who explained the reduction of BI by the lower degree of attenuation of vaccines, in which the virus was capable to destroy B-lymphocytes present in BF, leading to reduction in their size. This result was in agreement with that of Mazariegos *et al.*, (1990); Van den Berg (1991); Eterradossi *et al.* (1992); Amer *et al.*, (2007) who reported that intermediate strains of IBDV vaccines were sufficient to induce a significant reduction in BI.

The splenic atrophy may occurred due to the severe necrosis in the splenic tissue. This result was in agreement with that of McFerran, (1993) who reported that the reduction of spleen index was due to germinal centers and perivascular sheaths necrosis.

As shown in Table (1) also, there was a significant reduction ($P < 0.05$) in the thymic index in group 1 (2.740 ± 0.097) at 28th day of age.

The reduction of the thymic index clearly correlated with decreasing of thymus weight. However, thymus atrophy may occur due to severe depletion and necrosis of lymphocytes in the thymus tissue. The result of thymic index of the present study was in agreement with that of Hedayati *et al.*, (2005) who stated that thymic index of vaccinated groups which administrated at 16th day of age with intermediate vaccine was less than that of control group which indicated thymic atrophy induced by intermediate vaccine.

The result of histopathological changes in this study was in agreement with those of Ezeokoli *et al.*, (1990) who evaluated the histopathological modifications of BF associated with poultry vaccination against IBDV, and described severe lesions in the bursa between three and seven days after vaccination. The results of this study were also in agreement with those of Rautenschlein *et al.*, (2003) who reported that commercial broilers vaccinated with IBDV vaccines of different virulence intermediate and intermediate plus showed variable severity of lesions and the severity of the lesions of the intermediate plus vaccine was of score 4.

Al-Sereah, (2007) observed that chickens vaccinated with intermediate vaccine (Cevac) at 14th day showed more severe lesions such as hyperemia, dilatation of blood vessels and secondary follicular proliferation in BF 3 days post vaccination.

This difference of histopathological changes might be attributed to the number of passages which were used for attenuation. The breed of broiler chickens may have a role in this process. Khan *et al.*, (2007) noticed that local wild strain of IBDV at fifteenth passage causes a loss of complete ability to produce histopathological lesions in BF.

Depending on the virulence of the live attenuated viruses, some vaccine strains can cause bursal damage (Mazariegos *et al.*, 1990).

Splenic congestion and hyperplasia of reticulo-endothelial cells around the adenoid sheath artery in the vaccinated birds of this study were also observed previously by Ley *et al.*, (1983); Nunoya *et al.*, (1992); Hassan *et al.*, (1996), while necrosis was found by Okoye and Uzoukwu (1981); El-Manakhly and Bekheit (1992).

It is worth mentioning that, the above mentioned histologic alterations which observed in the thymus were previously demonstrated by Sharma *et al.*, (1989); Goodwin *et al.*, (1996); Goodwin and Hafner (1997); Shaban (2004); Amer *et al.*, (2007).

The results of the histopathological examination of the bursa, spleen and thymus gland in the vaccinated birds of present study indicated that live IBDV vaccines were enough to cause some pathological lesions in the lymphoid organs. These results were in agreement with those of Thornton and Pattison, (1975) Ide, (1979); Thangavelu *et al.*, (1998); Mona, (2002); Amer *et al.*, (2007) who mentioned that IBD vaccines induced pathological lesions in the lymphoid organs of chickens vaccinated with intermediate vaccines.

Study of the pathogenicity of four commercial IBD vaccines showed considerable variation in their pathogenicity. Based on the recorded findings, the intermediate vaccines can be divided into 3 pathogenic categories "highly, moderate and low pathogenic". According to the results of group 4, this vaccine considered to be more pathogenic than other intermediate vaccines. In conclusion, vaccine D proved to be more pathogenic than A, B, and C.

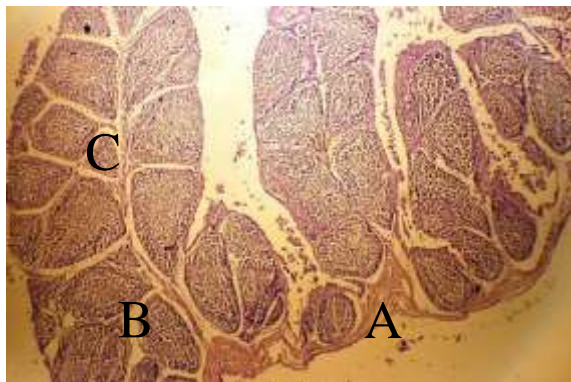


Fig (1): Cross histological section in BF (group1) at 17th day showed: A- Edematous area , B- Increased interfollicular space C-Follicular atrophy- (Score 3). H&E stain, 80x

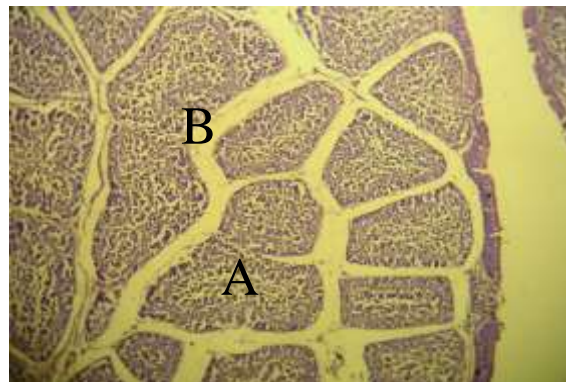


Fig (2): Cross histological section in BF (group2) at 28th day showed: A- Depletion of lymphocytes in medullary area B-Severe increasing of interfollicular spaces -(Score 4). H&E stain, 200x

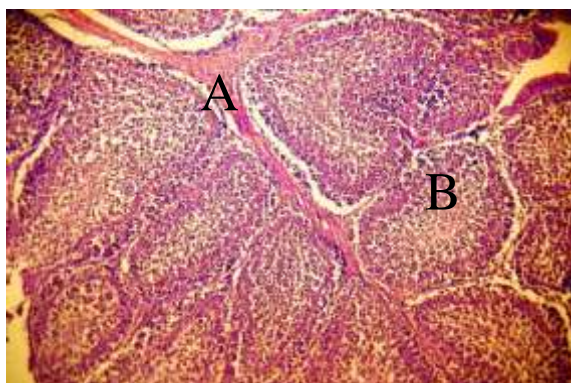


Fig (3): Cross section of BF (group3) at 17th day showed: A- Hyperplasia of interfollicular septa B- Depletion of lymphocytes - (Score 2). H&E stain, 250x

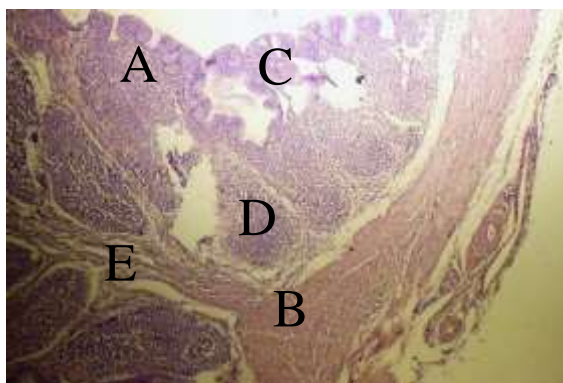


Fig (4): Histological cross section in BF (group4) at 28th day showed: A- Hydropic degeneration in parenchymal and epithelial cells B- Hyperplasia C- Cystic degeneracy D-Follicular atrophy E-Edematous area -(Score 4). H&E stain, 100x

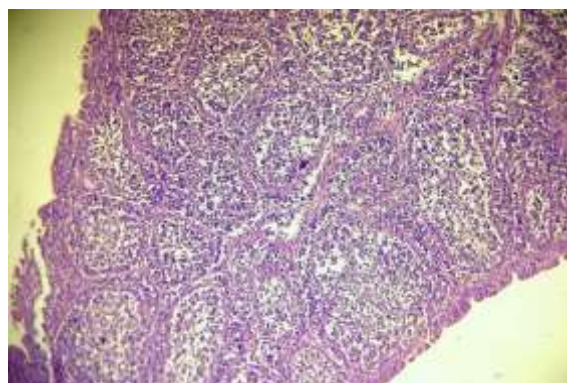


Fig (5): Histological cross section in BF (control) at 17th day showed normal structure of bursal tissue. H&E stain, 200x

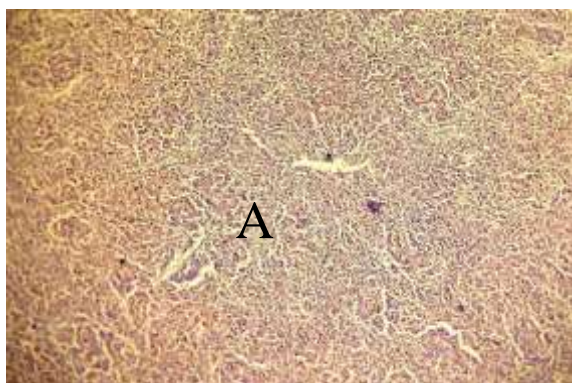


Fig (6) Cross section of spleen (group1) at 17th day showed A. Necrosis and lymphocytic depletion in the center of organ. H&E stain, 100x

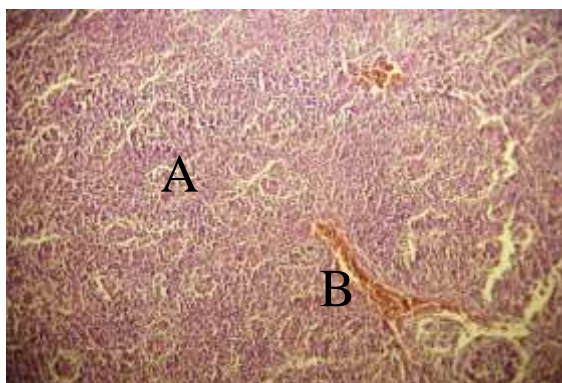


Fig (7):Cross section of spleen (group2) 28th day showed A- Depletion of lymphocytes, B- Sinusoidal congestion. H&E stain, 100x

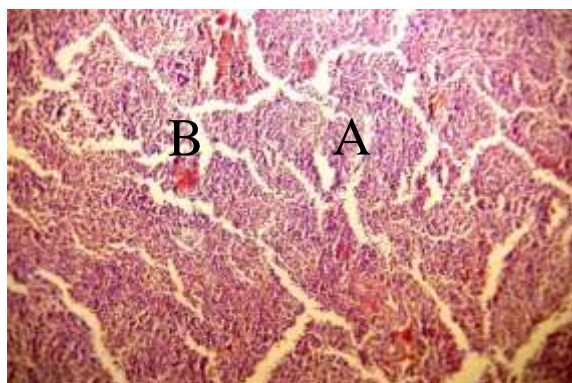


Fig (8): Spleen. Cross section (group3) at 28th day showed A- Follicular atrophy, B-sinusoidal congestion. H&E stain, 100x

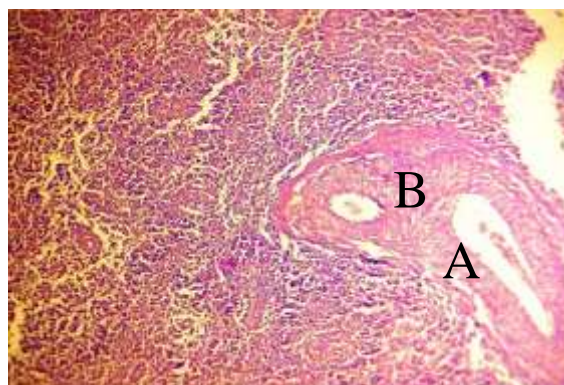


Fig (9): Spleen (group4) at 17th day showed A- Thickening of the wall of the blood vessels B- Hydropic degeneration in the epithelial layer. H&E stain, 100x

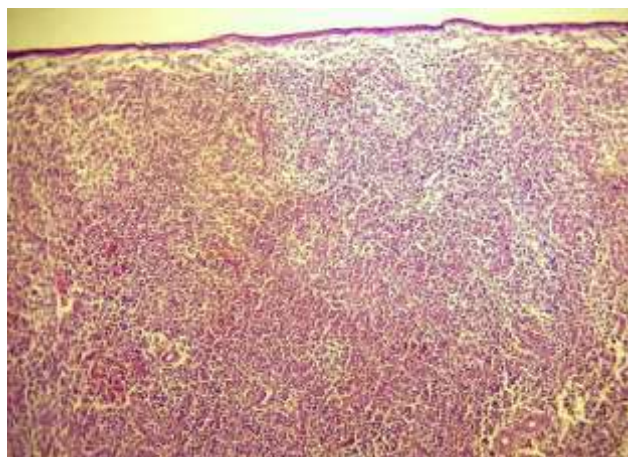


Fig (10):Spleen. Histological cross section (control) at 17th day showed normal splenic structures. H&E stain, 80x

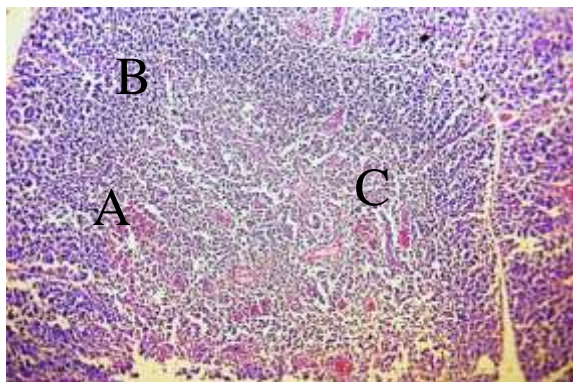


Fig (11):Thymus .Cross section , (group1) 17th day showed: A-Congestion in the medulla B- Severe lymphocytic depletion C- Multifocal areas of hemorrhage in medulla. H&E, 100x

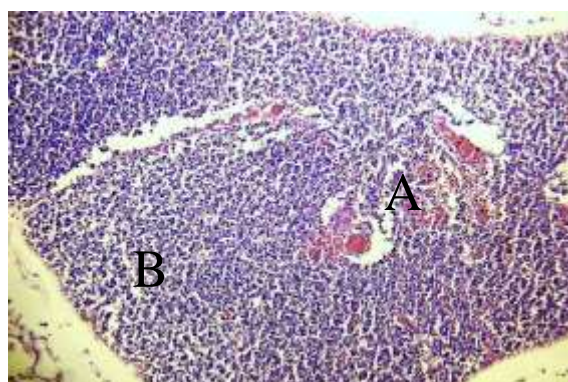


Fig (12):Thymus. Cross section (group2) 28th day showed A- Depletion of lymphocytes in medulla B- Diffuse hemorrhage. H&E stain, 100x

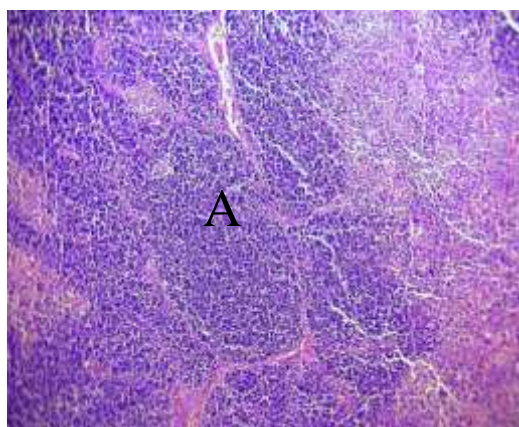


Fig (13):Thymus. Cross section (group3)17th day showed A- Mild depletion of lymphocytes. H&E stain, 80x

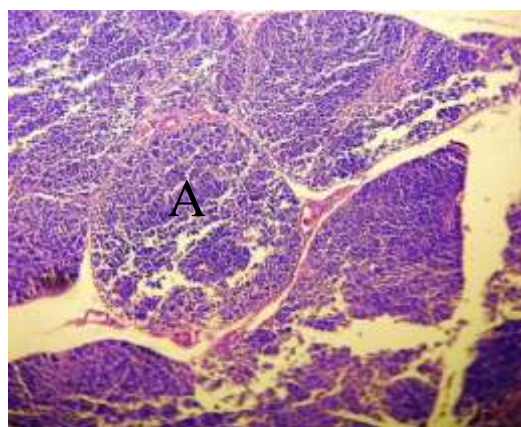


Fig (14):Thymus. Cross section , (group4) 28th day showed A- Thymic follicular atrophy due to depletion of thymic lymphocytes. H&E stain, 80x

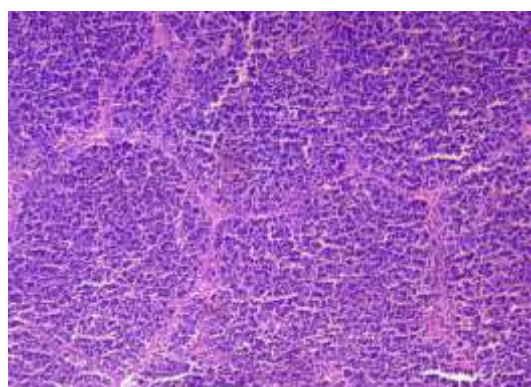


Fig (15):Thymus. Cross section , unvaccinated (control) 28th day showed normal thymic structures. H&E stain, 100x

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