

## Histological effect of infectious bursal disease virus on bursa of fabricia, Thymus gland and bone marrow tissues with monitoring of virus antibodies level during experimental infection on broiler chicken.

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### Abstract:

Infectious bursal disease virus infect wide range of poultry flocks across the world causing variable ranges of economic losses come from direct or indirect mortality through immune suppression by invading of B cell tissues, this study designed to show the effect of oral and ocular inoculated virus collected and processed from infected farms on bursa of fabricia, Thymus gland, bone marrow tissues with monitoring of virus antibody titer before and after age of inoculation (21 day old), the study showed different degrees of damage varies between tissues including degeneration, necrosis, congestion and edema, the study also showed that the maternal antibodies was high at the first day of hatching and the decrement of antibody titer happen directly after virus inoculation then increase in antibodies titer started gradually after two weeks of inoculation.

### Introduction:

Infectious bursal disease (IBD) or Gumboro disease is an acute, highly contagious viral disease of young birds characterized mainly by severe lesions in the bursa of Fabricius followed by immunosuppression. (1) & (2). The disease causing destruction to the lymphoid cells especially in bursa of fabricia (3). Within thymus gland from which the virus was frequently isolated the virus causes severe lesions (4). Bone marrow lesions including lysis, depletion and other different pathologic changes depend on severity of infection were described within many researchers results (5).

### Materials and Methods:

Fifty non-vaccinated chick was used in experimental infection for monitoring antibodies titer and histological effect before and after oral and ocular inoculation (at day 22) of the virus suspension, the inoculum prepared from bursa gland, the gland was collected from infected farms around Tikrit city and stored in deep freezer before thawed and 1 gm of bursa with equal amount of sterile sand with 9 ml of phosphate buffer saline to make a suspension, centrifuged at 700 xg for 15 min (three time) to take supernatant fluid, penicillin and streptomycin was added (10,000 IU of penicillin and 10 mg of streptomycin) to the suspension then incubated for 30 minutes and the suspension was kept at -20°C until time of use, Bursa of fabricia, thymus gland and bone that suggested being a model for histological diagnosis after experimental infection were collected with aseptic technique and immersed in 10% formaldehyde 24 hour then washed under running water for one hour and kept in 70% ethanol until time of histology except bone samples which treated with

5% hydrochloric acid one week before it kept in 70% ethanol, the stains prepared according to Drury *et al.* (1988) (6), tissues sections was stained according to procedure of Humason (1979) (7), blood was collected from the right jugular vein without using of coagulant, then samples were centrifuged at 252 xg 15 minutes to isolate the serum that stored at -20°C till use for serological purposes, the procedure was according to Symbiotic Kit protocol with using of (BIO-TEK ELX800) ELISA reader and washer, the optical density used for calculating antibodies titer by using the equation below:

$$Sp = \frac{\text{Sample Absorbance} - \text{Average Normal Control Absorbance}}{\text{Corrected Positive Control Absorbance}}$$

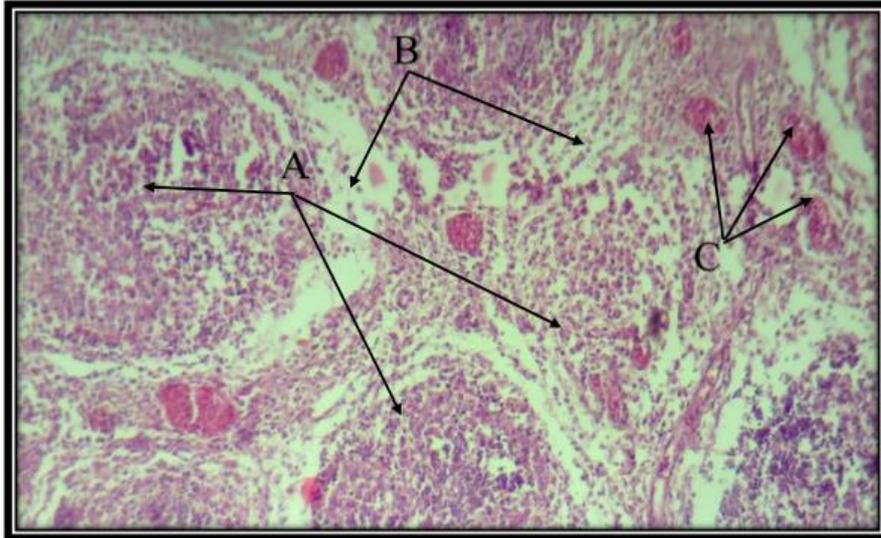
IBD ELISA titer calculated using the following suggested equation using Microsoft Excel software:

$$\text{LOG}_{10} \text{ TITER} = (1.172 \times \text{LOG}_{10} Sp) + 3.614$$

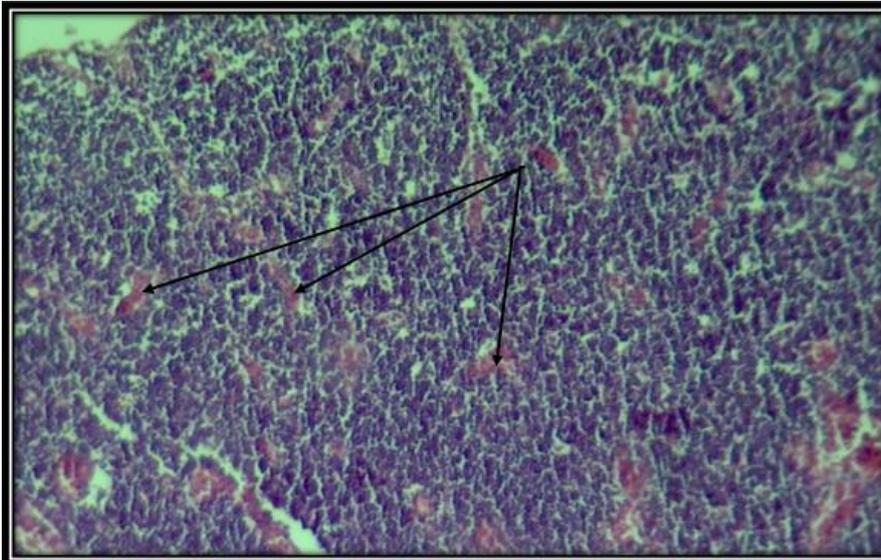
$$\text{TITER} = \text{ANTILOG OF LOG}_{10} \text{ TITER}$$

### Results:-

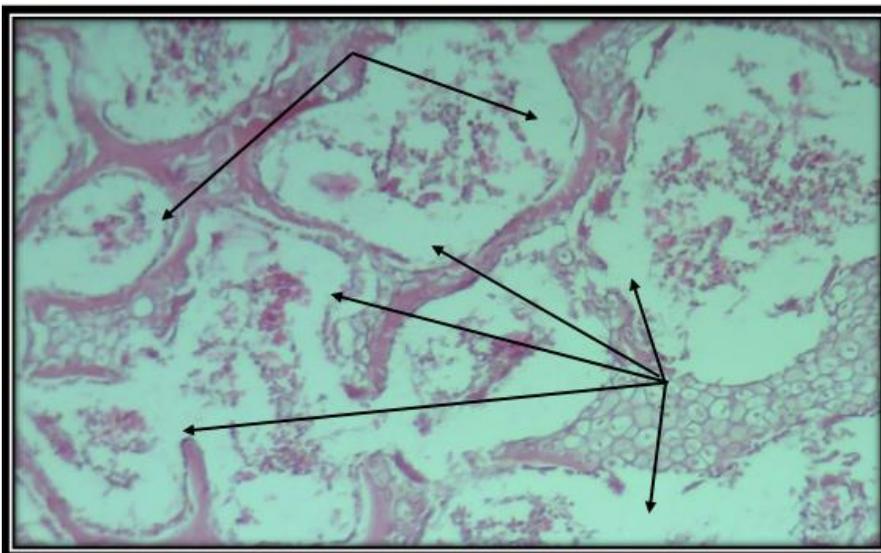
The collagen bundles of the Bursa of fabricia capsule were small and scattered, in between the bundles there were different types of the inflammatory cells the follicles of the bursa showed variable degrees of depletion, congestion and edema (figure 1), the tissue of the Thymus gland show many areas of hyperplasia in germinal center and different areas of congestion (figure 2). bone marrow show cellular depletion especially in the paratrabeular area (figure 3), Chicks maternal antibody titer level was high at one day old which decreased at 21 day after hatching, before antibody titer returned to increase after one week of oral and ocular inoculation (histogram 1 and table 1).



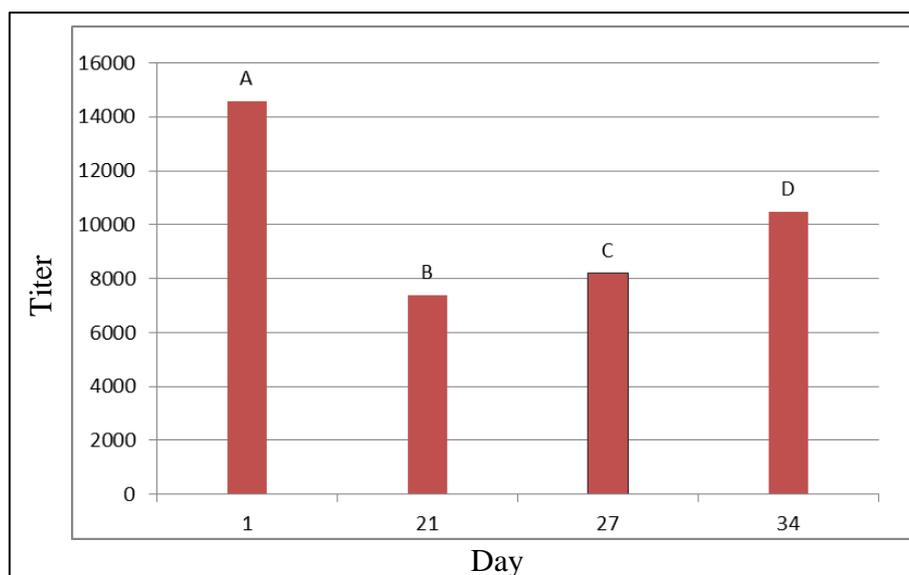
**Figure (1)** Histological section of bursa of fabricia shows follicular depletion (A) , edema (B) and blood congestion (C) (H & E X 100)



**(Figure 2)** Histological section of thymus gland shows congested area in the tissue (H & E X100)



**(Figure 3)** Histological section of bone marrow shows cellular depletion (E & H X400 ).



**Histogram (1) Level of maternal antibody for experimental chicks, A and B represent antibody titer before inoculation, C and D represent antibody titer after inoculation (C & D) experimental infection**

**Table (1) antibodies mean titer during experiment with maximum and minimum value**

Age per day	Mean	Maximum value	Minimum value
1	14569.28	16885.92	1513.24
21	7379.363	13221.60	181.76
27	8202.124	15281.99	3246.50
34	10489.09	15424.96	4031.65

### Discussion:

The histological lesions of the bursa agree with Inoue *et al.* (1999) (8) were characterized by follicular lymphoid necrosis and depletion, heterophil and macrophage infiltrations, Stromal fibrosis and infiltration of plasma cells, depleted lymphoid follicles occupied with epithelial reticular cells, bursa lymphoid follicles hypotrophy, necrosis and heterophils invasion (9) was suggested to be caused by IBDV replication in immature B lymphocytes which is serve as a virus target (10).

Lysis of bursal lymphocytes has been caused by both necrosis and apoptosis as it showed by Tanimura & Sharma, (1998) (11); Vasconcelos & Lam, (1995) (12), while Ahmed & Akhter (2003) (13) show that the bursal epithelium appeared hyperplastic with folded and thickened and undifferentiated epithelial cell layer between the cortex and medulla, the differences in results in the present study and other studies could be result from the different stages of disease and tissue repair mechanism which intern it subject to many self and environmental conditions but not because differences between field isolates and vaccinal samples (14).

Thymic lesions in the present study agree with Inoue *et al.* (1999) (8) results about degeneration and necrosis stages, the lysis of thymic lymphocytes could be caused by highly virulent and some virulent reference strains of IBDV directly even without virus replication (10), Inoue *et al.*, 1994 (15); Tanimura *et al.*, 1995 (16).

There is no differences in thymic tissue damage during IBDV infection become to be a fact as it showed by Ignjatovic *et al.* (2004) (5) who explained that the endemic classical IBD viruses present in Australia cause minimal changes in the thymus and spleen while there are no marked differences in the severity of lesions between Australian classical and Australian variant strains. this study agree with Ignjatovic *et al.* (2004) (5) also in bone tissue, they show that the bone marrow exhibited severe lysis and depletion of heterophil myelocytes and lysis of bone marrow cells is faster at early stage following IBDV not like thymus gland, there are distinct differences in pathologic changes between the bursa and the bone marrow between two isolates, there are distinct differences in pathological changes between the bursa and the bone marrow of two isolates, these differences come to diminish with highly virulent strains of IBDV and severe bone marrow lesions as well as bursal lymphoid necrosis appear (17).

Bone tissue damage was suggested to be caused by myelocytes apoptosis in this case the cell show morphologic characteristics of apoptosis (18), (19), (20).

Because of low blood and lymphatic supplementations to the bone the amount of virus in the bone marrow become small rather than it cause a severe damage when compared with the amount of antigen in other tissues (16). Indirect ELISA test antibodies titer (histogram 1) that gives protective immunity agree with Michell *et al.* (2009) (21) study

that showed the progeny of the vaccinated breeders would have high titers of passive antibody at hatching, Van der Berg and Meulemans (1991) (22) showed the same results, commercial hatchery hatching eggs from broiler breeders vaccinated with oil emulsion this vaccine stimulate passive IBDV-specific serum neutralizing antibody and provided protective antibody level to the next progeny (23), this maternal immunity supposed to be the protective shield that prevents disease appearance (24), declined titers (histogram 1) after one week and during life of chicks are agreed with Ahmed & Akhter (2003) (13) they showed that the level of antibodies maternal immunity was maintained up to 7 days of age then declined subsequently at day 28 of age, the difference appeared in the results of the above studies and our study could be due to the difference in the initial titer of IBD in chicks (Ahmed & Akhter, 2003) (13) or

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due to the presence of aflatoxin in feed (25) which have a detrimental effect on humeral and cell mediated immune response, leading to fulminating disease outbreaks even after vaccination (26) . decrease or lack of the heat-stable serum factors that inhibit the infection by IBDV also decrease the IBDV antibodies level (27), many other different reasons could cause an effective role on IBDV antibodies level and chick immune system as a whole impairment of lymphokines production and antigen processing by macrophages could be one of them (28), antibody titer increment at day 27 and 34 in histogram (2) after challenge in chicks agrees with Ceica and Vasiliu (2008) (29); Ahmed and Akhter (2003) (13) even the fact that showed IBDV viruses continued to change and may circumvent the immune system that could decrease antibodies level (30).

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## التأثير النسيجي لفايروس التهاب جراب فابريشيا على غدة فابريشيا , غدة التوتة ونخاع العظم مع رصد مستوى الاجسام المناعية المضادة للفايروس خلال إصابة تجريبية على الدجاج اللاحم

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### الملخص

يصيب فايروس التهاب جراب فابريشيا المعدي طيف واسع من حقول الدواجن حول العالم مؤدي الى ظهور مستوى مختلف من الخسائر الاقتصادية ناجمة عن هلاك الطير بصورة مباشرة او بصورة غير مباشرة بواسطة تثبيط الجهاز المناعي للطير والنتاج عن استهداف الفايروس لأنسجة خلايا (ب) المناعية، صممت هذه الدراسة لإظهار تأثير الفايروس المقطر في العين والدم بعد جمعة ومعاملته من حقول مصابة على نسيج غدة فابريشيا , غدة التوتة و نخاع العظم ومراقبة تأثير الفايروس على معدل الاجسام المناعية المضادة للفايروس قبل وبعد إعطاء الفايروس (بعمر 21 يوم)، وبينت الدراسة حصول تأثير مرضي تتباين شدته بتباين الانسجة ويشمل انحلال, تنخر, احتقان و وذمة, وان ان معدل الاجسام المناعية للأفراخ بعمر 1 يوم عالي ينخفض مباشرة بعد إعطاء العالق الفايروس ثم يعود ليرتفع تدريجاً بعد أسبوعين من إعطاء العالق الفايروس.