

## **Interference of Infectious Bronchitis Virus Vaccine with Immune Response induced by Infectious Bursal Disease Virus Vaccine in Broiler Chicks.**

**تداخل فايروس لقاح التهاب القصبات المعدي مع الاستجابة المناعية لفايروس لقاح التهاب جراب فابريشيا المعدي في فروج اللحم.**

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### **Summary**

The study was designed to investigate the immunological interference or impact of live attenuated infectious bronchitis disease vaccine with live attenuated infectious bursal disease vaccine in broiler chicks. One hundred and seventy commercial broiler chicks were selected at one day of age, the maternal antibody titer was measured, then chicks divided randomly in to four equal groups; G1 vaccinated with IB and IBD, G2 vaccinated with IB, G3 vaccinated by IBD and G4 (Control group).

The results of indirect ELISA revealed significant differences that G1 gave mean values of antibody titer of IBDV lower than G3, no significantly differences of bursa of Fabricius index, the result of lymphocytes count showed a significant differences which was high in G3 and a significant lower in G1, also the heterophils count showed a significant differences, which was high in G1 and significant lower in G3, the microscopically examination of Harderian Gland of G1 and G2 showed few plasma cells infiltration, and severe congestion of the blood vessels with inflammatory cells infiltration and few fibrous connective tissue proliferation, while harderian gland of G3 showed marked lymphocyte and plasma cell infiltration. It is concluded that the (IB) vaccine interfere or has impact on immune response of (IBD) vaccine.

### **الخلاصة**

صممت هذه الدراسة للتحري عن التداخل المناعي لفايروس لقاح التهاب القصبات المعدي مع الاستجابة المناعية للقاح التهاب جراب فابريشيا المعدي في فروج اللحم في محافظة السليمانية. استخدم في التجربة أفراخ دجاج لحم تجارية بعمر يوم واحد وعددها (170) طير. تم التضحية ب(30) طير عشوائيا لقياس المناعة المتأتية من الامهات لكلا المرضين بعمر يوم واحد باستخدام اختبار الأنزيم المناعي المتمز غير المباشر (Indirect ELISA) وكانت نتائج المناعة الأمية جيدة لكلا المرضين. قسمت بقية الأفراخ عشوائيا وعددها (140) طير الى اربعة مجاميع متساوية بواقع (35) طير لكل مجموعة, ثلاثة مجاميع عدت كمجاميع معاملة بينما تركت المجموعة الرابعة بدون تلقيح وعدت كمجموعة سيطرة. لقحت المجموعة الأولى (G1) بلقاح التهاب القصبات المعدي ولقاح التهاب جراب فابريشيا المعدي, بينما المجموعة الثانية (G2) لقحت ضد مرض التهاب القصبات المعدي فقط. واما المجموعة الثالثة (G3) حيث تم تلقيحها ضد مرض التهاب جراب فابريشيا المعدي فقط, وكانت طريقة التلقيح في جميع المجاميع عن طريق التقطير في العين, وتركت المجموعة الرابعة (G4) بدون تلقيح وعدت كمجموعة سيطرة.

اظهرت النتائج الفحوصات المصلية للاستجابة المناعية لمرض التهاب جراب فابريشيا المعدي فروق معنوية بين المجموعتين (G1 & G2) باستخدام اختبار (Indirect ELISA) حيث كانت واطنة في المجموعة الاولى وعالية في المجموعة الثانية, لم تكن هناك فروق معنوية في معدل منسوب جراب فابريشيا للمجاميع المعاملة, وكانت هناك فروق معنوية في عدد الخلايا اللمفاوية حيث كانت عالية في المجموعة الثالثة ومنخفضة في المجموعة الاولى. على عكس نسبة الخلايا المتغيرة حيث كانت عالية بشكل معنوي في المجموعة الاولى ومنخفضة في المجموعة الثالثة, واطهرت التغيرات النسيجية المرضية المجهرية لغد هاردر للمجموعة الاولى والثانية ارتشاح قليل للخلايا البلازمية واحتقان شديد للاوعية الدموية اضافة الى ارتشاح الخلايا

الالتهابية في الغدة، اما المجموعة الثالثة اظهرت ارتشاح واضح جدا للخلايا البلازمية والمفاوية. نستنتج من ذلك ان لقاح التهاب القصبات المعدي تتداخل او تؤثر على الاستجابة المناعية للقاح التهاب جراب فابريشيا المعدي في فروج اللحم .

## **Introduction**

Infectious bronchitis is an acute and highly contagious disease of respiratory and urinary systems of chickens (14). It was isolated in Iraq for the first time in broilers and layers (2) also isolated from broilers in Mosul province (1). In the last period, the disease distributed in different governorates of Iraq, diagnosed with indirect ELISA technique, presences of respiratory signs resemble the signs of Newcastle disease (respiratory form) (23), and the last publication recorded the disease in Sulaimanayah province (27), it was thought that they were due to vaccination with unsuitable strain of IB, all the IB vaccines which used commercially and are not matched with serotypes that exist in layers and broilers. Consequently, this may emerge new variant strains of IB viruses.

Epizootological and economical importance of this disease due to many variant serotypes and strains reported in the world (19).

Russell, 1996 referred that infectious bronchitis viral vaccine had immunosuppressive effect to local immunity to Newcastle disease viral vaccine, that IBV induced transient necrosis of plasma cells in the Harderian gland. Moreover there was primarily confirmed information about immune interference of infectious bronchitis viral vaccine with immune response of Newcastle disease viral vaccines (34, 4, and 33).

the viral interference phenomena can occur among different serotypes of the same virus, for example infectious bursal disease virus with intermediate and pathogenic strains (7), It can also occur between different viruses, as between infectious bronchitis virus and Newcastle disease virus (5), the occurrence of interference among vaccines can be promoted by the competition among the vaccinal viruses on the same receptors (12).

Since the Infectious Bursal Disease is a highly contagious viral and acute infection with tropism for lymphoid tissue (17), which is one of the important diseases in Iraq epidemiologically and economically, therefore, the decision of this study is the possibility of presence of interference or impact of infectious bronchitis virus vaccine with immune response of infectious bursal disease virus vaccine in commercial broiler chicks.

## **Materials and Methods**

IBV Massachusetts H120 strain and IBDV (D 78) strain (Intervet – Holland) Used in the study, titration of the Vaccines Viruses has been done according to (25).

One hundred and seventy commercial broiler chicks were selected in this study at one day of age. Thirty chicks were sacrificed randomly for detection of maternal derived antibodies against IBDV and IBV in their sera at one-day age, by using indirect ELISA technique the rest chicks (140 chicks) were divided into four equal groups, thirty five chicks for each. Three groups were considering as treated groups numbered as G1, G2 and G3 while the last group (G4) was considered as a control group and as following:-

- 1) First group: The chicks of this group were vaccinated with a live attenuated Infectious Bronchitis disease vaccine at the following ages (1, 10,) days, as well as they were vaccinated by a live attenuated infectious Bursal disease vaccine at 11 days of age.
- 2) Second group: The chicks of this group were vaccinated by a live attenuated Infectious Bronchitis disease vaccine at the following ages (1 & 10) days.
- 3) Third group: The chicks of this group were vaccinated by a live attenuated Infectious Bursal disease vaccine at the age (11) day.

The route of vaccination was done by ocular route for all vaccinated groups.

- 4) Forth group: (Control group) this group has been left without any vaccination.

The Parameters used in the study were Indirect Enzyme Linked Immunosorbent Assay (ELISA), bursal index, blood picture (differential count H\L) and histopathological study of Harderian gland.

### **Calculation of Bursal Index**

The Bursa/body weight ratio was calculated according to the formula of (26).

$$\text{1- B/B weight index} = \frac{\text{Bursa/body weight ratio of infected birds}}{\text{Bursa/body weight ratio of control birds}}$$

### **Differential counting of WBC`s:-**

The method was mentioned by (10). One drop of blood for each treatment was dripped on the slide then this drop was spread with one stroke and after getting dried; a slide was dyed with Wright stain and left 10 min. then washed with tap water.

### **Results and discussion**

Results of titration of vaccines viruses were conformed with the dose of the company manufactured and the results of maternal derived antibody (MDA) titer at one day of chicks' age recorded good mean titer against IBDV and IB, these due to activation of the mucosal immunity in the reproductive tract that cause direct secretion of antibodies into the eggs (21), also the MDA titers of the control group, against IBDV and IBV at day (30) of the chicks' age decreased to ~zero this indicated that there was no field or vaccinal viruses' challenges during the experiment, whereas the mean values of Antibody titer against IBD at 30 days of age by the indirect ELISA test recorded in two groups (G1, G3) were significantly differences (It was found that G1 gave mean values of antibody titer lower in a comparison with G3(table 1).

**Table (1) Values of ELISA test against (IBD) at (30) day of age.**

<b>Groups</b>	<b>Mean Ab titer± SE</b>
<b>G1</b>	<b>5348± 0.1 A</b>
<b>G3</b>	<b>5687± 1.0 B</b>
<b>G4</b>	<b>3± 92 C</b>

Values are mean ± SE .Values followed by different letters on the table are significantly different (P<0.05).The symbol (G) represents the group.

The explanations of these differences were IBV induced a decrease in the capacity of immune response of gland of Harder (GH) (28), and the stressed factors produced post-vaccination with IB lead to worse effect on immune status, and this explanation is in accordance with the study of (36 and 11), in addition, IBV vaccine induced a transient necrosis of plasma cell in the gland of harder (31) this followed by less lachrymal IgA (16). Also the interpretation for these finding was that the vaccination of chicks by more than one vaccine during a short period lead to immune stress which enhanced interleukin-1 and corticosteroid level (30) and lead to lymphoid cell destruction with reduction of antibody production (22).

The results of bursa of Fabricius index at 14 and 30 days of chicks' age were not significantly differences (table 2).

**Table (2) Values of Bursal index at (14<sup>th</sup> & 30<sup>th</sup>) day of age.**

<b>Groups</b>	<b>Mean± S .E 30<sup>th</sup> Day</b>	<b>Mean± SE 14<sup>th</sup> Day</b>
<b>G1</b>	<b>0.245± 0.117 A</b>	<b>0.254± 0.117 A</b>
<b>G2</b>	<b>0.251± 0.141 A</b>	<b>0.26± 0.141 A</b>
<b>G3</b>	<b>0.291± 0.023 A</b>	<b>0.261± 0.023 A</b>
<b>Control</b>	<b>0.238± 0.155 A</b>	<b>0.248± 0.155 A</b>

Values are mean ± SE .Values followed by similar letters on the table are not significantly different (P<0.05).The symbol (G) represents the group.

The results of these finding are agreed with (32), there was absence of any effect on bursal index in the vaccinated groups, this indicate that the vaccine being safety according to (24, 3).

While the result of lymphocytes count showed a significant difference between the groups at 30 days which was high in (G3) and a significant lower in (G1), also the heterophils count was showed a significant difference between the groups, which is high in (G1) and significant lower in (G3) (table 3).

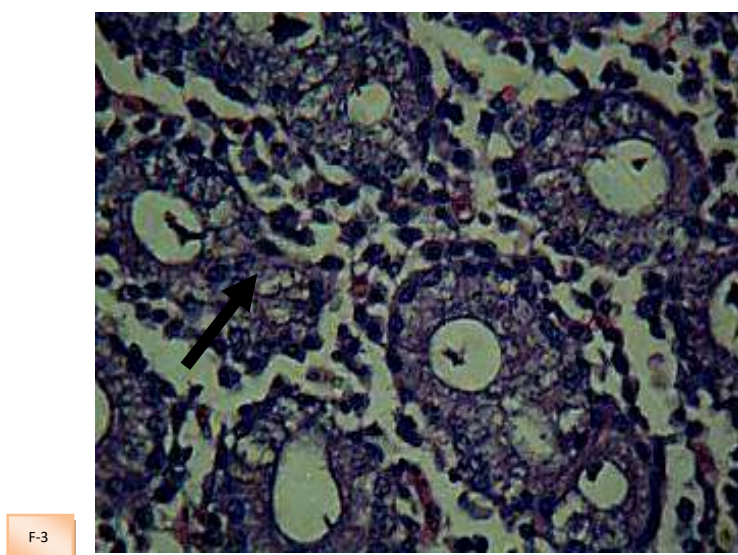
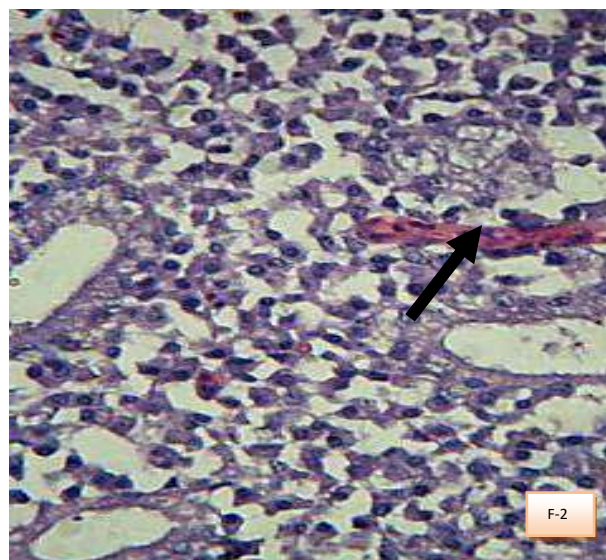
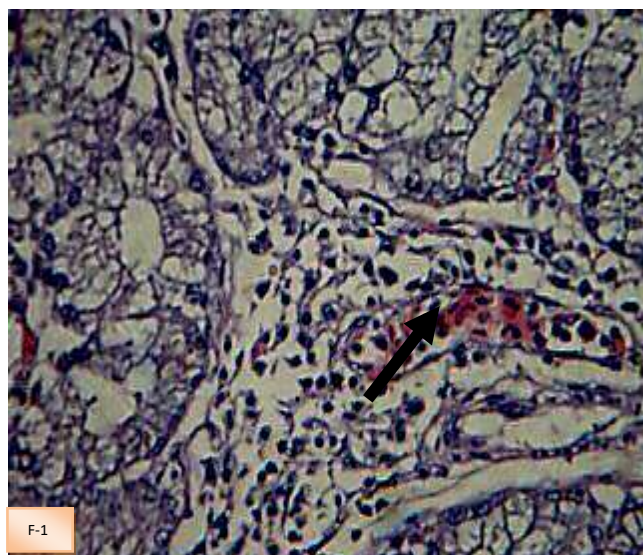
Table (3) Values of lymphocytes & heterophils count at (30) day of age.

<b>Groups</b>	<b>Mean lymphocytes count % <math>\pm</math> S. E</b>	<b>Mean heterophils count % <math>\pm</math> S. E</b>
<b>G1</b>	<b>60 <math>\pm</math> 0.894 A</b>	<b>35 <math>\pm</math> 0.660 A</b>
<b>G2</b>	<b>68 <math>\pm</math> 0.409 C</b>	<b>26 <math>\pm</math> 0.50 C</b>
<b>G3</b>	<b>70 <math>\pm</math> 0.707 C</b>	<b>24 <math>\pm</math> 0.690 C</b>
<b>G4 (Control)</b>	<b>63 <math>\pm</math> 0.860 B</b>	<b>29 <math>\pm</math> 0.844 B</b>

Values are mean  $\pm$  SE .Values followed by different letters on the table are significantly different ( $P < 0.05$ ).The symbol (G) represents the group.

The interpretation for this finding is that the multivaccination stress lead to loss of cortical lymphocytes (apoptosis of cortical lymphocytes) and followed by removal of apoptotic debris by macrophages, this finding is accordance to (18) who referred that Stress causes elevated circulating levels of glucocorticosteroids then to loss of cortical lymphocytes, these changes are usually reversible on removal of the stress factor. also a variety of factors and conditions result in an alteration in the cellular density and cellular composition, such as the age and genetic factors , the adequacy of nutrition and the stress levels which leads to these changes (25).

It is obvious that the results of increasing the heterophils as a reaction resulting by virus invasion for lymphocytes in bursa and increasing destroying them. Such changes were noticed by (15) who mentioned the increasing of heterophils and decreasing in lymphocytes, he explained the decrease in lymphocyte to virus increasing and damaging these lymphocytes. In addition the heterophils are considered as the ruling kind within granulation leukocytes in cases of acute infectious immune response in birds, also, they are very effective cells in phagocytes, and its number rises in blood in infection cases as a result of increasing its release from bone marrow (8). Its inflammatory ability was increased to disease causatives as a part of inflammatory response (6).and regarding to the results of microscopically examination of Harderian Gland showed few plasma cells infiltration between glandular ducts and there is severe congestion of the blood vessels with inflammatory cells infiltration in the interstitial tissue of Harderian gland of (G1) as well as few fibrous connective tissue proliferation around blood vessels as shown in (fig 1), and the histological examination of (G2) showed lesion similar to that reported in (G1) but more intense of mononuclear cells infiltration and dilatation of the glandular ducts with mucin and mononuclear cells in the lumens as appeared in (fig 2), the histopathological finding in these two groups occurred due to effect of IBV and in accordance with (31) who referred that infectious bronchitis viral vaccine induced transient necrosis of plasma cells in the Harderian gland also IBV induced a decrease in the capacity of immune response of Harderian gland (28), and the stressed factors produced post-vaccination with IB lead to worse effect on immune status, and this explanation is in accordance with the study of (26 and 11), while harderian gland of (G3) showed marked lymphocyte and plasma cell infiltration in the interstitial tissue with congestion of the blood vessels and dilatation of glandular ducts and presence of mucin in their lumens as shown in (fig 3) this is the normal stimulation and reaction post vaccination.



**Histopathological section of harderian gland in chicks, F-1 showed congestion of blood vessels and inflammatory cells infiltration with fibrous connective tissue proliferation around blood vessels, F-2 severe mononuclear cells infiltration between mucosal gland with dilation of glandular ducts with mucin in their lumen, F-3 showed severe plasma cells and lymphocytes with congestion of blood vessels (H&E  $\times 40$ )**

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