

**A STUDY OF EFFECT OF INFECTION BURSAL DISEASE VIRUS AND
VACCINE ON THE HUMORAL IMMUNE RESPONSE TO NEWCASTLE
DISEASE VACCINE IN BROILER CHICKS**

By

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Abstract

The immune response on vaccination against Newcastle disease in broiler chicks is frequently reduced in case of infection with Gumboro disease or vaccination against Gumboro disease. The study aimed to study the immunological interference in broilers between infectious bursal disease viruses, live attenuated infectious bursal disease vaccine and live attenuated Newcastle Disease vaccine. One hundred and forty commercial broiler male were selected in this study at one day of age. Forty chicks were sacrificed randomly to measure maternal antibody titer against Newcastle disease virus and infectious bursal disease virus in their sera. One hundred chicks were divided randomly in to four equal groups. First group , Second group , Third group, were vaccinated against Newcastle disease virus vaccine LaSota strain by drinking water at (1,10,20,30) days of age. Furthermore, Second group was inoculated by infectious bursal disease virus at (28) day of age. Third group was vaccinated against infectious bursal disease virus vaccine (Winterfield 2512-G-61) strain by drinking water at (7, 14, 21, 28) days of age. The results obtained that there were a significant increase ($P<0.05$) in the mean titer of first group in HI test at (23rd) and (33rd) day of age. The results of indirect ELISA showed that there were a significant increase ($P<0.05$) in the mean titer of antibodies against Newcastle disease virus of first group at (35) day of age. The results revealed a significant decrease ($P<0.05$) in the mean values of HI test against Newcastle disease virus of the second and third groups at (23rd) and (33rd) day of age.

It is concluded that the humoral immune response against Newcastle disease virus was influenced directly due to the immunological interference of field and vaccinal strains of infectious bursal disease virus.

Key words: IBD virus, IBD vaccine, ND vaccine, Immunological interference

Introduction

Infectious bursal disease virus (IBDV) is an acute and highly contagious viral disease of young chicken. It belongs to the genus Avibirnavirus, family Birnaviridae [1]. IBDV is the causative agent of acute or immunosuppressive disease in chickens because of the resulting morbidity and mortality as well as the immune suppression. The disease affects primarily bursa of Fabricius and other lymphoid organs to lesser degree. The virus could be classified into two serotypes serotype 1 and serotype 2. Serotype 1 strains are pathogenic, with the target organ being bursa of Fabricius (BF) while studies on serotype 2 strains demonstrated that they do not cause disease or protect against infection [2]. This project aimed to study the effect of immunological interference between infectious bursal disease virus and live attenuated infectious bursal disease vaccine on the humoral immune response to Newcastle disease vaccine in broilers.

MATERIALS AND METHODS

Experiment Design

One hundred and forty commercial broiler males at one day of age from breeder of Arbor Acres (Iraqi Company for production and Marketing of meats, Al-Kalis poultry fields) were obtained to carry out this experiment. Forty chicks were chosen randomly and blood samples collected by sacrificing of these chicks at one day of age for demonstration of indirect ELISA in order to measure the derived maternal antibody titer against Newcastle disease virus and infectious bursal disease virus in their sera. The rest chicks were divided randomly into four equal groups (25 chicks for each). First group, Second group and Third group, were vaccinated against Newcastle disease virus vaccine LaSota strain by drinking water at (1, 10, 20, 30) days of age. Furthermore, Second group was inoculated by infectious bursal disease virus at (28) day of age. Third group was vaccinated against infectious bursal disease virus vaccine (Winterfield 2512-G-61) strain by drinking water at (7, 14, 21, 28) days of age.

Collection of bursae samples

Infected bursae were collected from an outbreak of infectious bursal disease at a local poultry farms in Diyala governorate. Complete history of outbreak was taken. These samples were stored at $-20\text{ }^{\circ}\text{C}$ till used.

IBD Field virus isolation of titration

An IBD virus was isolated from infected bursae according to the method mentioned of Reddy et al ^[3]. The embryo lethal dose (ELD_{50}) of the isolated virus was tittered by making of series of ten fold serial dilution from 10^{-1} to 10^{-10} in sterile phosphate buffered saline (PBS) saline. The diluted virus was inoculated by dropping of (0.1) ml per egg on chorioallantoic membrane (CAM) of nine day old chick embryo. After inoculation all the eggs were sealed with melted wax and were re-incubated at $37.5\text{ }^{\circ}\text{C}$ for seven days. Inoculated eggs were candled daily. Mortality during first 24 hours was discounted as non-specific. The (ELD_{50}) was $10^{6.3}$

Blood samples Collection of serum titration

At (13, 23, 33) day of ages, five blood samples were collected randomly from chicks of treated groups plus control group. At (35) day of age, ten blood samples were collected randomly from chicks of treated groups plus control for demonstration of indirect ELISA to measure the antibodies against Newcastle disease virus in their sera.

Procedure of vaccination by drinking water

1) Newcastle vaccine:

The live attenuated Newcastle vaccine "LaSota" strain has been used to vaccinate the chickens of all treated groups by drinking water method at (1,10,20,30) day of age.

2) Infectious bursal disease vaccine:

The live attenuated infectious bursal disease vaccine "Winterfeild 2512 – G-61" strain has been used to vaccinate the chickens of third group by drinking water method at (7, 14, 21, 28) day of age.

Statistical analysis:

The data obtained in this study was analyzed using the two-way and one-way analysis of variance and least significance differences to determine the differences among groups ^[4].

RESULTS

Results of values of indirect ELISA test at one day of age.

The results showed a significant differences ($P < 0.05$) among all the tested chickens at one day of age. The results revealed a highest level of the mean value of derived maternal antibodies (DMA) against Newcastle disease virus at one day of age, whereas the derived maternal antibodies (DMA)titer against infectious bursal disease virus showed the lower mean value as shown in table (1).

Table (1): Values of indirect ELISA test to all groups (randomly) at one day of age.

Agent	Mean	S . E
ND.V	6430.2	± 356
IBD.V	4413.75	± 268.5

Results of values of HI test at (13th) day of age.

The results in table (2) revealed a significant differences ($P < 0.05$) of the mean values of HI test at (13th) day of age among all groups in which the results showed a significant differences in G1 , G2 and G3 in compared with control group.



Table (2): Values of HI test at (13th) day of age.

Group	Mean	S .E	L.S.D
First group	166.4 a	± 38.5	88.3
Second group	115.2 b	± 37.4	
Third group	128 b	± 35.1	
Control	49.6 b	± 21.8	

Values are mean ± SE "Standard Error" .Values followed by different letters on the table are significantly different (P<0.05).L.S.D means Least Significant Differences. The letters (a and b) that differ vertically indicate to statistical significant variations (P<0.05).

Results of values of HI test at (23rd) and (33rd) day of age.

The results revealed a significant differences (P<0.05) among all the groups of HI test at (23rd) and (33rd) day of age as shown in table (3) and table (4). First group gave the highest mean value of antibody titer of HI test in comparison with second, third and finally control groups at (23rd) and (33rd) day of age.

Table (3): Values of HI test at (23rd) day of age.

Group	Mean	S .E	L.S.D
First group	153.6 a	± 25.6	38.4
Second group	64 b	± 17.6	
Third group	32 b	± 8.7	
Control	17.6 b	± 5.8	

Table (4): Values of HI test at (33rd) day of age.

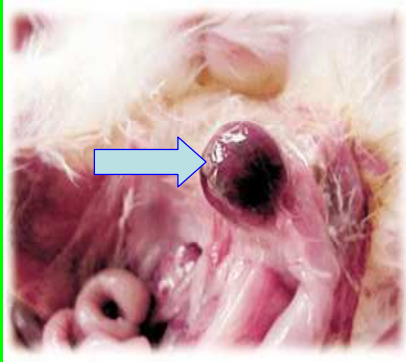
Group	Mean	S .E	L.S.D
First group	115.2 a	± 31.4	32
Second group	14.4 b	± 4.6	
Third group	16 b	± 4.3	
Control	4.8 b	± 1.9	

Results of indirect ELISA test against (NDV) at (35) day of age

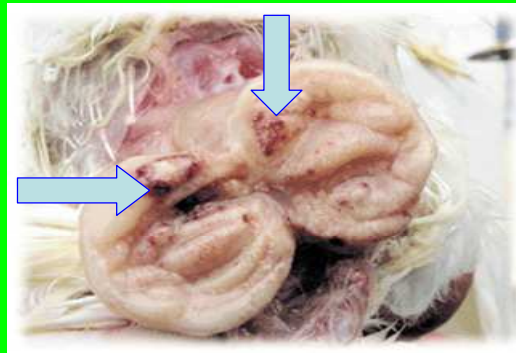
The recorded results in table (5) showed a significant differences ($P < 0.05$) among the mean values of all groups at (35th) day of age in which first group gave the highest mean value (13524.7) in comparison to the rest groups. It was found that third group showed significantly increase ($P < 0.05$) in the mean value in comparison with second and control group.

Table (5): Values of Antibody titer of indirect ELISA test against (NDV) at (35) day of age.

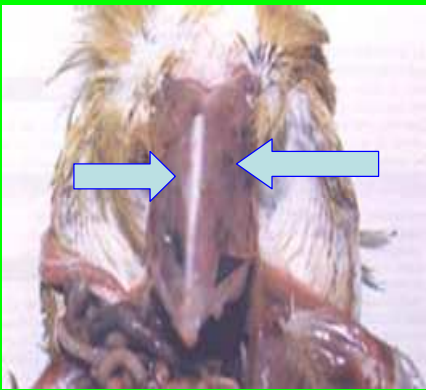
Group	Mean	S .E	L .S .D
First group	13524.7 a	± 475.8	855.4
Second group	8966.7 c	± 273.8	
Third group	11972.2 b	± 518.5	
Control	1929.9 d	± 18.3	



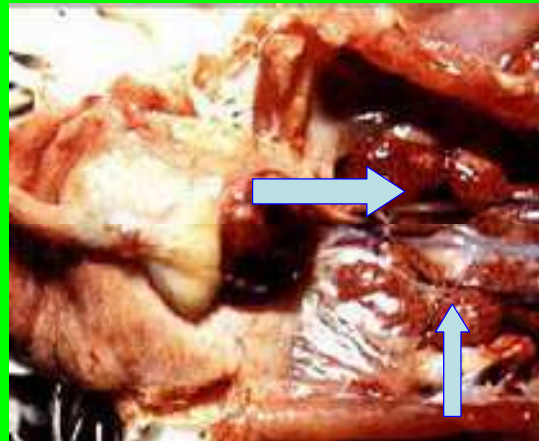
Picture (1) explaining infection of bursa of fabracious post challenge by IBD virus. (Notice bursal hyperatrophy "pointer").



Picture (2) explaining infection of bursa of fabracious post challenge by IBD virus. (Notice bursa enlargement and hemorrhage "pointer")



Picture (3) showing infection of muscles of chest of chickens post challenge by IBD virus. (Notice the hemorrhage of thorasic muscles "pointer")



Picture (4) showing infection of kidneys of chickens post challenge by IBD virus. Notice enlargement of kidneys "pointer")

DISCUSION

The results of the mean value of derived maternal antibodies (DMA) against Newcastle disease virus of indirect ELISA test showed a significant differences ($P < 0.05$) among all the chickens at one day of age. The results revealed a highest mean value of derived maternal antibodies (DMA) against Newcastle disease virus then against infectious bursal disease virus in their sera. This is ascribed to previous vaccination of breeders against Newcastle Disease (ND)

and against infectious bursal disease (IBD) or breeders might be infected by ND or (IBD) previously. This is in agreement with many studies ^[5, 6].

The results revealed a significantly increase ($P < 0.05$) of the mean values of hemagglutination inhibitory antibodies of HI test of first, second and third groups at (13th) day of age. This is may attributed to presence of high levels of antibodies in breeders due to immunization of these breeders against Newcastle Disease virus, therefore these maternal antibodies against Newcastle Disease virus transmitted from breeders to baby chicks via passive immunity on high levels also. These findings agreed with many studies ^[7, 8]. The results revealed a significant decrease ($P < 0.05$) of second and third groups of HI test values at (23rd) and (33rd) day of age. This is ascribed to significant depression of primary antibody of chickens to ND vaccine when administered one week after IBD infection or vaccination. This agrees with the findings of ^[9]. This could be due to the effect of the virus on the lymphoid organs, such as the bursa of Fabricius of the infected birds. The virus causes necrosis of the lymphocytes in the medullary area of the lymphoid organs resulting in the suppression of both humoral and cell mediated immune responses ^[10, 11]. The reduced capacity of response can decrease the level of antibody response to NDV when the birds are vaccinated to IBDV and NDV in the same time. This confirms the findings of ^[12, 13, 14]. Other studies ^[4, 6, 10] referred a similar situation to IBDV, showing that there was a decrease of plasma cell quantity in the gland of Harder during infectious bursal disease virus infection of 3-week-old broiler chickens; this might induce deficiency of local immunity in the paraocular region and upper respiratory tract associated with IBD. The results revealed a significant decrease ($P < 0.05$) of second and third groups of indirect ELISA test against (IBDV) at (35th) day of age. This is may be ascribed to the immunological interference between infectious bursal disease virus and Newcastle Disease virus which occur due to the initial infection tropism of these viruses. This is agreed with the findings of ^[15].

CONCLUSION

It is concluded that infectious bursal disease virus serotype II and live attenuated infectious bursal disease vaccine effect directly on the humoral immune response that induced against live attenuated Newcastle Disease vaccine "LaSota" strain. This might cause through reduction of the hemagglutination inhibitory antibodies against Newcastle disease virus because

of the immunological interference between infectious bursal disease virus, live attenuated infectious bursal disease vaccine and finally live attenuated Newcastle Disease vaccine if chickens were vaccinated together.

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((دراسة تأثير فيروسات مرض التهاب جراب فابريشيا الخمجي ولقاح الكمبورو على الاستجابة المناعية الخلطية للقاح النيوكاسل في فروج اللحم)).

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الخلاصة

الاستجابة المناعية للتلقيح ضد مرض النيوكاسل في فروج اللحم غالباً ماتقل في حالة الإصابة بمرض الكمبورو أو التلقيح ضد مرض الكمبورو. هدفت الدراسة تأثير التداخل المناعي الحاصل بين فيروسات مرض الكمبورو ولقاح الكمبورو على الاستجابة المناعية للقاح النيوكاسل عترة. استخدم في التجربة ذكور افراخ دجاج لحم تجارية سلالة (اربور اكرس) بعمر يوم واحد فقط وعددها (140) طير. تم التضحية ب(40) طير عشوائياً بعمر يوم واحد فقط لقياس الاضداد الامية ضد فيروسات مرض النيوكاسل وللتحري عن الاضداد الامية ضد فيروسات مرض الكمبورو في مصولها. قسمت بقية الافراخ عشوائياً وعددها (100) طير الى اربعة مجاميع متساوية. لقحت المجاميع الاولى والثانية والثالثة بلقاح النيوكاسل عترة لاسوتا بطريقة ماء الشرب عند الاعمار (30,20,10,1) يوم. علاوةً على ذلك حقنت المجموعة الثانية بفايروسات الكمبورو ولقحت المجموعة الثالثة بلقاح الكمبورو بينما تركت المجموعة الرابعة كمجموعة سيطرة بينت النتائج تفوق معنوي مهم احصائياً على مستوى (5%) في معدل معيار الاضداد المثبطة للتلازن الدموي ضد فيروسات مرض النيوكاسل في مصولها للمجموعة الاولى مقارنة مع المجاميع الباقية عند الاعمار (23,33) يوم. أثبتت الدراسة تفوق معنوي مهم احصائياً على مستوى (5%) في معدل معيار الاضداد المثبطة للتلازن الدموي ضد فيروسات مرض النيوكاسل في مصولها للمجموعة الاولى مقارنة مع المجاميع الباقية في اختبار الانزيم المناعي المتمز غير المباشر عند عمر (35) يوم فقط. بينت النتائج بانخفاض معنوي على مستوى (5%) في معدل معيار الاضداد ضد فيروسات مرض النيوكاسل لافراخ المجموعتين الثانية والثالثة عند عمر (33,23) يوم. نستنتج من ذلك، أن فايروسات مرض الكمبورو ولقاح الكمبورو أثرت بصورة مباشرة على المناعة الخلطية المتحفزة ضد لقاح من خلال تقليل معيار الاضداد المثبطة للتلازن الدموي ضد فايروسات مرض النيوكاسل LaSota مرض النيوكاسل عترة نتيجة التداخل المناعي بين العترة الحقلية والعترة اللقاحية لفايروسات مرض الكمبورو.

الكلمات المفتاحية : فايروسات الكمبورو ، لقاح الكمبورو ، لقاح النيوكاسل ، التداخل المناعي .