INTERFERING OF INFECTIOUS BRONCHITIS VACCINE (MA5 AND 4/91) STRAINS ON THE IMMUNE RESPONSE OF NEWCASTLE AND AVIAN INFLUENZA VACCINES IN MALE LAYERS

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ABSTRACT

The aim of this study was to investigate the immunological interference of Infectious Bronchitis vaccine (IBV) MA5 and 4/91 strains on immune response to Newcastle (ND) and Avian Influenza (AI) vaccines by studying the humeral immunity in layers. A total of 90-day-old male layer chicks were assigned into three equal treatment groups as follow: G1 chicks were vaccinated against AI at 2nd days and against ND at 2, 10, 20 and 30 days of age. G2: chicks were vaccinated as in G1 and vaccinated against IB at 10 and 30 days of age. G3: chicks in this group were left without vaccination as a control group. the result showed that the antibodies titers against ND and AI were high in G1 in contrast with G2 and control group at 20 and 45 days of age. A key finding from these results revealed that vaccination of chicken by using of live attenuated IBV leading to immunological interference and immune stress which negatively affect on the immune response against ND and AI vaccines.

INTRODUCTION

The IB, ND and AI are an important viral diseases result in considerable economic losses to the poultry industry worldwide Cavanagh (4), most characterized by respiratory signs, poor weight gain, reduced feed efficiency, a drop in egg production and quality and mortality Kapczynski et al. (8). Control of these diseases depend on biosecurity procedures and vaccination Tu et al. (13). Attenuated live and inactivated oil-emulsion vaccines are generally effective in controlling the disease. However, IB outbreaks may occur despite the use of the vaccine. Moreover, new viral strains continually emerge and mutation and recombination occur frequently in attenuated live vaccines Jia et al. (7).

In order to reduce costs of vaccination, the use two or three vaccines simultaneously became a common practice in poultry production, such as a combined vaccine against ND and IB. Earlier studies have reported that IB virus interferes with the immune response against ND virus Cardoso et al. (12). Also productivity losses related to viral interference in broiler flocks, cell culture and embryonating chicken eggs Brown (3), Smith (11) Yachida (14).

Infectious bronchitis disease is one of the diseases that reported in flocks of layers and broilers in many governorates of Iraq Azab et al. (1). However, it was not achieved to prepare the suitable live attenuated IB vaccine which match with the same serotypes of virulent field strains. All the IB vaccines, which using are commercially imported and are not match the serotypes that exist in layers and broilers.

The purposes of this study were to determine the effects of live attenuated IB vaccine on humeral immune response of ND and AI vaccines in layer by using a different vaccination programs. To assess the humeral

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immunity, the Haemagglutination Inhibition test (HI), Enzyme Linked Immunosorbent Assay (ELISA), Bursal and Spleen indices were employed.

MATERIALS AND METHODS

Animals and Experimental Design

Ninety-day-old, male layer chicks "Lohmann brown strain" were purchased from Al-Hadetha company. Upon arrival, ten chicks were sacrificed and blood samples were collected for HI test in order to measure the Derived Maternal Antibody Titer (DMAT) against NDV in their sera; also indirect ELISA test was performed to detect DMAT against NDV, IBV and AIV. The rest 90 chicks were weighed and divided randomly into three equal groups including two treated groups. First group G1 was vaccinated by inactivated oil based AI and ND vaccine (0.5ml) intramuscularly at 2nd days of age. Then after, vaccinated by live attenuated ND vaccine (clone 30) by eye drop at 10, 20 and 30 days of age.

Second group G2 was vaccinated by the same vaccination programmed of G1, with live attenuated IB vaccine at 10 and 30 days by MA5 strain and 4/91 strain respectively and third group had been left without any vaccination as control group. Blood samples were randomly collected from ten birds from each group at 20 and 45 days for HI and ELISA test to determine the antibody titer of NDV and AIV. Then after, ten chickens were sacrificed at day 40 for bursa and spleen index. Treated groups were given live attenuated IBD vaccine (228E) at 16 days of age. The chickens were raised according to routine management practice. All nutrients including water were supplied *ad libitum*.

Hemagglutination Inhibition (HI) Test

The HI-Ab titers to NDV which was done according to the method describe by Allan and Gough (1974).

Enzyme Linked Immunosorbent Assay (ELISA)

A Synbiotics Kits (commercial Corporation) of indirect ELISA were carried out at Uruk Veterinary Center and the Group.

Bursa and Spleen Indices

chickens were individually weighed from each group to determine their body weight. Ten birds from each group at day 40 were sacrificed via cervical dislocation. Following a thorough visual appraisal, the bursa Fabricious and spleen were immediately removed, dry and individually weighed. Since substantial lymphoid organ weight change was anticipated, their indices were calculated Sellers et al. (10). Using these formula: Organ index = organ weight (g)/BW (g) x 100.

Statistical Analysis

The data were analyzed using one-way analysis of variance. Differences between means were determined using Tukey tests in which the significance level was designated at $P<0.05$.

The Results of maternal derived antibody (MDA) titer, the highest mean values of MDA titer against NDV followed by IBV, whereas the MDA titer against AIV showed the lower mean value as shown in Table 1.

Table 1: Values of maternal Ab titer of ELISA test against ND, IB, and AI at 2nd day of age

Agent	Mean ± SD
ND	4602 ± 606
IB	3729 ± 1187
AI	938 ± 216

The values of Ab titer of HI against NDV at 20 and 45 days of age are shown in Table 2. A trend of an increase of Ab titer was seen in all groups except that in control group during the course of the experiment. Commencing from day 20 the layers from the G1 has the highest (P<0.05) values. At these instants, Ab titer in the G2 was significantly (P<0.05) different from the control.

Table 2: Values of Ab titer (Mean ± SD) of HI against NDV at 20 and 45 days of age

Groups	HI at 20 days of age	HI at 45 days of age
G1	69 ± 12.89^{a}	256 ± 57.45^{a}
G2	25 ± 4.36^{b}	85 ± 13.45^{b}
Control	9 ± 1.22^{c}	1.3 ± 1.33^{c}

[a, b, c] Values having similar superscript between column did not differ at (P<0.05).

Similarly, an increasing pattern of Ab titer was seen in all groups as time advances except that in control group (Table 3). However, the layers from the G1 has the highest (P<0.05) values when compared with the other groups. At these instants, Ab titer in the G2 was comparable from the control at 20 days of age.

Table 3: Values of Ab titer (Mean ± SD) of ELISA test against NDV at 20 and 45 days of age

Groups	Titers at 20 days of age	Titers at 45 days of age
G1	2186 ± 351.8^{a}	16490 ± 2193^{a}
G2	706 ± 142.3^{b}	5380 ± 242^{b}
Control	278 ± 42.5^{c}	27 ± 27.4^{c}

[a, b, c] Values having similar superscript between column do not differ at (P<0.05).

However, highest (P<0.05) levels Ab titer against AIV in the ELISA were seen in the G1 throughout the entire experiment period (Table 3). Significantly (P<0.05) higher Ab titer against AIV in the G2 than the control group was recorded at 20 and 45 days of age.

Table 4: Values of Ab titer (Mean ± SD) of ELISA test against AIV at 20 and 45 days of age

Groups	Titers at 20 days of age	Titers at 45 days of age
G1	598 ± 57.6^{a}	964 ± 105^{a}
G2	374 ± 16.6^{b}	479 ± 72^{b}
Control	146 ± 65.9^{c}	100 ± 64^{c}

[a, b, c] Values having similar superscript between column do not differ at (P<0.05).

The bursa and spleen indices are shown in Table 5. Although the control group showed the highest (P<0.05) bursal index compared with the other groups, fluctuations were seen in the spleen index. The highest (P<0.05) spleen index was only seen in the G2.

Table 5: Relative bursa and spleen weight to live body weight of male layers at 40 days of age

Groups	Bursa index	Spleen index
G1	0.230 ± 0.016^{b}	0.095 ± 0.005^{b}
G2	0.195 ± 0.004^{c}	0.130 ± 0.018^{a}
Control	0.305 ± 0.015^{a}	0.086 ± 0.007^{b}

[a, b, c] Values having similar superscript between column do not differ at (P<0.05).

The Discussionis is Monitoring the level of serum Ab is commonly used as an index to assess the amount of protection induced by vaccination. There is considerable confusion in the commercial broiler industry relating to vaccination with ND, AI and IB vaccines. Questions arise over route of vaccination, age at vaccination, doses of vaccine to administer per bird, mixing of ND, AI and IB vaccines or other vaccines, vaccine interference and reactions, use of live vaccines, strains or serotypes of virus and others. ND, AI and IB vaccination should provide protection to a flock against field virus challenge without causing excessive reactions. An integration or relationship must determine during the vaccinating.

The maternal antibody titers (Table 1) can interfere with the response to the first and second vaccine stimulus promoting the neutralization of the first vaccination and a different response to the second one, according to high or low maternal antibodies. Our data revealed that, in almost all instances the G1 has a highest ($P<0.05$) levels of Ab titer against NDV, AIV (Tables 2, 3 and 4) in comparison with G2 and control groups during the course of the experiment. This was interpreted that G1 was vaccinated by AI and ND vaccine only, but the G2 was vaccinated by the same vaccination programme of G1, with live attenuated IB vaccine at 10 and 30 days, that mean the IBV in this group has interfered with NDV and AIV immunity response, which consequently produce low levels of NDV and AIV Ab titers, finally a weak humoral immunity response were archived. The interference between IBV virus and other respiratory viruses (NDV and AIV) occurs because both of them infect initially the epithelial cells of respiratory tract and also this might be due to increased histopathologic lesion in the trachea of vaccinated groups, administration of mixed vaccine has demonstrated to induce deciliation, hyperplasia, hyperemia and some lesions in the tracheal epithelial mucosa Gelb et al. (6), Nakamura et al. (9). As a consequence, interference can result in high levels of stress, low production and an insufficient immunity against field strains of NDV and AIV.

The lymphoid organs indices are useful indicators, and have been assessed in many immune responses. The bursa of Fabricius and spleen are important lymphoid organs which involved in the development and differentiation of B or T lymphocytes Eerola et al. (5). Chicks when vaccinated by more than one vaccine during a short period lead to immune stress on the bursa, this process struggled the function of B-cell inside the bursa consequently this will negatively affect on the production of antibodies, thus lead to bursal atrophy and finally embarrassed the humoral immune response. T-cells are present in a large numbers in the spleen. These cells undergo non-specific stimulation for cell-mediated immunity in a wide range by using different antigens. T-cells would be activated later on and finally increase the mean value of the spleen index in chicken of G2 Bankowski (2). The fluctuation in lymphoid organs (bursa and spleen) in the G2 (Table 5) denotes a negative effect on the production of antibodies and was due to invoked by the IBV instilled. These results matched with the results of HI and ELISA. According to the present results we recommended the farmers do not used the vaccines against IB (MA5 and 4/91 strains) since these strains had an immunosuppression effect to the humoral immunity against ND and AI. More studies should be performed to better understand the factors that lead to the interference of IBV on the immune response against ND and AI vaccine.

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تداخل لقاح التهاب القصبات المعدي (عترة 4/91 and MA5) مع الاستجابة المناعية للقاحي النيوكاسل والانفلاونزا في ذكور الدجاج البياض

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الملخص

تهدف هذه الدراسة إلى بيان التداخل المناعي للقاح التهاب القصبات المعدي الحي مع الاستجابة المناعية الخلطيه للقاحي النيوكاسل والانفلاونزا في أفراخ الدجاج البياض. ولبيان هذا التداخل استخدم اختباري أثباط التلازن الدموي والإنزيم المناعي الممتز غيرالمباشر وكذلك معدل وزن جراب فابريشا والطحال إلى وزن الجسم. استعمل في هذه الدراسة تسعون من ذكور أفراخ الدجاج البياض بعمر يوم واحد قسمت هذه الأفراخ إلى ثلاث مجموعات متساوية لقحت المجموعة الأولى (G1) بلقاح الأنفلونزا + النيوكاسل بعمر يومين، كما لقحت بلقاح نيوكاسل الحي المضعف فقط في عمر 10، 20، 30 يوماً على التوالي. اما المجموعة الثانية (G2) فقد لقحت بالبرنامج اللقاحي نفسه للمجموعة الأولى + لقاح التهاب القصبات المعدي الحي المضعف عند عمر10 و30 يوماً على التوالي وتركت المجموعة الثالثه بدون تلقيح وعدت كمجموعة سيطرة. بينت النتائج وجود مستوى مناعي جيد في المجموعة الأولى ضد مرض النيوكاسل والأنفلونزا مقارنه مع المجموعة الثانية ومجموعة السيطرة للأيام 20 و45 على التوالي. نستنتج من هذه الدراسة إن للقاح التهاب القصبات المعدي تأثير سلبي في مستوى الأجسام المناعية للقاحي النيوكاسل والانفلاونزا.

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