Immunosuppressive Properties of Infectious Bronchitis Vaccine4/91 strain against different programs and method of vaccination against Newcastle disease in Broilers

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Abstract

Infectious bronchitis virus (IBV) primarily induces a respiratory disease in broilers causing important economic losses in poultry industry. The aim of this study was to investigate the immunosuppressive properties of live IB vaccine on the different programs and method of vaccination against Newcastle disease (ND) in broilers. A total of 125 one-day-old broiler chicks were assigned into five equal groups. At day one G1 and G2 groups were vaccinated with a live (ND, IB) vaccine by dipping the head in the vaccine and intra-cloacae method respectively. Afterward, G3 and G4 groups were vaccinated with a live ND vaccine alone by the same method of G1 and G2. At the same time all these 4 groups were vaccinated by subcutaneous injection (S/C) with inactivated oil based vaccine ND. The G5 group was left without vaccination as a control group. After that, all treated groups were vaccinated with a live ND by spray method at day 10 of the age. The immunosuppressive effects of this IB virus was evaluated by Haemagglutination inhibition test (HI), and challenged by virulent ND virus. Bursa and Spleen indices were estimated in all groups. The results showed that the antibody titers against ND was higher ($P < 0.05$) in G3 and G4 in compared with G1, G2 and control groups at 20 and 30 days of age. However, there was no significant difference of bursa index and spleen index in all groups. Challenge test showed no mortality in the 3rd group while 10% in the 4th group, 85% in the 1st group and 90% in the 2nd group. In conclusion it's revealed that the best method and program of vaccination which can protect against ND was applied in the 3rd group.

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.(2). Infectious bronchitis virus is the prototype species of the family *Coronaviridae*, more than 25 genotypes are distributed worldwide. IBV causes an acute highly contagious viral respiratory disease of chickens which is characterized by respiratory rales, coughing and sneezing .(7). Some of IBV strains replicate in the gastrointestinal tract, oviduct, kidney, and due to their nephropathogenic properties they have the potential to cause severe losses with up to 44% mortality.(7; 8). In other cases, infection of the proventriculus leads to 75% to 100% mortality in young

birds.(9). Newcastle disease is regarded throughout the world as one of the important diseases of poultry and other birds.(1). The mortality rates up to 100%, especially in unvaccinated flocks.(4). Because of the severe nature of the disease and the associated consequences, ND is included as an Office International des Epizooties, (OIE) list a disease and most countries, including all European Union countries, enforce statutory control measures in the event of outbreaks of disease. (5). Control of these diseases depends on biosecurity procedures and vaccination . 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. Earlier studies have reported that IB virus interferes with the

immune response against ND virus .(15;14). This study was conducted to evaluate the different methods and program to found the best solution of problem to ND infection,

and also to detect the immunosuppression caused by IB vaccine on immune response of ND vaccine.

Materials and Methods

Vaccines

Vaccines of ND commercial vaccine CL30, inactivated oil based vaccine of ND lasota strain, IB vaccine 491 strains, all vaccines supplied from Intervet Company.

Method of vaccination

Dipping method was applied by dipping head of birds in the vaccine solution of ND alone(1000dose per litter) for short period, or mixed vaccines IB and ND; both vaccines dissolved in one litter of distil water. Those vaccines were given at one-day-old with inactivate oil bused ND vaccine injected subcutaneously in the back region of the neck at a dose of 0.25ml according to the recommendation of Intervet Company.

Intra-cloacae method was applied by given the dissolved vaccines of ND alone(1000 dose per litter) or mixed ND and IB, both vaccines dissolved in one litter of distil water and by injection one ml of diluted vaccine in intra-cloacae at day-old with inactivate oil bused ND vaccine injected subcutaneously in the back region of the neck at a dose of 0.25 ml.

Spraying method was applied as a booster dose was given by spraying of Lasota vaccine at day10of age to all vaccinated groups.

Experimental animals and protocol

One hundred and twenty five one-day-old broiler(Cobb strain) chicks obtained from a local hatchery were kept at the Department of pathology and poultry disease, college veterinary medicine, University of Baghdad. Upon arrival, the chicks were divided randomly into five equal groups of 25 chicks each. First group G1 was vaccinated at one-day-old with IB and ND vaccine using dipping method and inactivated oil based vaccine S/C at one-day-old. Second group G2 was vaccinated at one-day-old with IB

and ND vaccine using intra-cloacae method and inactivated oil based vaccine S/C at one-day-old. Third group G3 was vaccinated atone-day-old by ND vaccine only by dipping of head and inactivated oil based vaccine S/C at one-day-old. Forth group G4 was vaccinated at day one old by ND vaccine using intra-cloacae method of vaccine and inactivated oil based vaccine S/C at one-day-old. Fifth group G5 was lifting un vaccinated as control group. Spraying method was applied as a booster dose was given by spraying of Lasota vaccine at day 10of age to all vaccinated groups.

Blood samples were randomly collected from ten birds from each group at 20 and 30 days for HI test to determine the antibody titer of NDV. (3). Chickens were individually weighed from each group to determine their body weight. Five birds from each group at day 20 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 .(10). Using these formula: Organ index = organ weight (g)/BW (g) x 100.Challenge test is applied 30 days and observation for 7 day after challenge to record clinical signs, gross lesion and mortality. The chickens were raised according to routine practice in terms of light and temperature. Water and feed were given *ad libitum*. The data were analyzed with SPSS 16.0 for Windows by using one-way analysis of variance and least significance differences to determine the difference between groups at level of $P<0.05$.

RESULTS

The values of HI titer in vaccinated groups against ND at 20 and 30 days of age showed significance of increase of Ab titers in compared with control group as illustrated in table (1). A trend of an increase of antibody

titer was seen in all groups except that in control group during the course of experiment. At these instants, Ab titer in the G3 and G4 was significantly ($P<0.05$) different from the G1 and G2.

Table (1) the value of Abs titer of HI against NDV at 20,30 days of age all experimental groups.

Groups	Mean± SE at 20 day of age	Mean ± SE at 30 day of age	Vaccines & Method of vaccination
G1	8.0± 2.80 ^b	10±1.7 ^b	Alive ND and IB, dipping & oily ND
G2	6.0±1.40 ^b	9.0±2.5 ^b	Alive ND and IB, intra-cloacae & oily ND
G3	18±5.25 ^a	40±8.0 ^a	Alive ND only, dipping & oily ND
G4	16±8.45 ^a	36±10 ^a	Alive ND only, intra-cloacae & oily ND
G5	3.5±0.50 ^c	0.0±0.0 ^c	Control

^{a, b, c} Values bearing similar superscript between column do not differ at ($P<0.05$).

The bursa and spleen indices are shown in table (2), the result showed significant differences between treated groups and

control group, highest mean values were recorded in treated groups while a less mean value in control group at day 20th of age.

Table (2) the results of bursa and spleen indices all experimental groups

Group	Bursa (Mean ± SE)	Spleen (Mean ± SE)
G1	0.150±0.012 ^a	0.125±0.030 ^a
G2	0.130±0.009 ^a	0.100±0.026 ^a
G3	0.140±0.160 ^a	0.130±0.015 ^a
G4	0.150±0.023 ^a	0.125±0.026 ^a
G5	0.110±0.019 ^a	0.080±0.029 ^a

Values bearing similar superscript between column do not differ at ($P<0.05$).

After challenge test, the first clinical sign of chicks was observed in the unvaccinated control group which characterized by the

respiratory sign, diarrhea, nervous signs and high mortality percentage was reached 100% table (3).

Table (3) Mortality rate after challenge test in all experimental groups

Group	Dead No.	Total No.	Vaccines & Method of vaccination	Mortality rate
G1	17	20	Alive ND and IB, dipping & oily ND	85%
G2	18	20	Alive ND and IB, intra-cloacae & oily ND	90%
G3	0	20	Alive ND only, dipping & oily ND	0%
G4	2	20	Alive ND only, intra-cloacae & oily ND	10%
G5	20	20	Control	100%

Nevertheless, high protective rate was recorded in G 3 and G4 were reached 100%, 90% respectively as illustrated in table (4).

Table (4) Protection rate after challenge test in all experimental groups

Group	Dead No.	Total No.	Vaccines & Method of vaccination	Protection rate
G1	17	20	Alive ND and IB, dipping & oily ND	15
G2	18	20	Alive ND and IB, intra-cloacae & oily ND	10%
G3	0	20	Alive ND only, dipping & oily ND	100%
G4	2	20	Alive ND only, intra-cloacae & oily ND	90%
G5	20	20	Control	0%

Discussion

The impact of IB vaccine on immune response on different methods of vaccination against ND was studied to show the important role of interference on humoral response against ND in layers. Our previous work was documented that IB impair the humoral immune response on ND vaccination titer in broilers .(6). The data of HI Ab titers revealed that there is a signification difference between the

vaccinated groups and control, and also showed that a significance difference in the immune response between G1,G2 groups (vaccinated with ND +IB) and G3,G4 groups (vaccinated with ND only) as illustrated in table (2). These data revealed that the higher HI Ab titer was due to avoiding gave live IB vaccine with ND vaccine in the same time to prevent the interferes of IB with ND vaccine.(12).determined that mixed IB,ND

vaccine lead to immune suppression and high mortality due to infection with ND. Bursa and spleen indices are useful indicators of immune response, the result showed that high values in vaccinated groups than that of control unvaccinated group. (11). The result of challenge test with virulent local isolated ND strain was evaluated in different vaccinated groups as shown in table(3). High mortality and sever clinical signs were appeared in unvaccinated control group, signs characterized by respiratory signs, diarrhea and nervous signs. Nevertheless, high protective rate in G3 and G4 against ND after challenge by virulent virus respectably 100%, 90% while G1, G2 had significantly low protection percentage 15%, 10%. The immune response of these different methods (dipping & intra-cloacae)

and programs (ND with IB and ND alone) create different stimulation in cell immunity and humoral immunity (local and systemic) leading to stimulation of high Abs titers as showed in groups G3 and G4 or low Ab titer as showed in groups G1 and G2. (12). According to the IB viral replication in the cecal tonsil and bursa Fabricius were affected as a result of the viremia due to live strain of IB vaccine which caused a pathological changes in these organs leads to destruction of lymphatic cell. (13). Also, the hardarian gland which is the target organ of viral replication was affected leading to necrosis of plasma cells, this change mean factors of immunosuppression of immune response as indicated by low Ab titer in G1 and G2 groups. (14).

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خاصية التثبيط المناعي للقاح التهاب القصبات المعدي عترة 4/91 في تقيم برامج وطرق لقاحية مختلفة ضد مرض النيوكاسل في دجاج اللحم

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الخلاصة

يعد التهاب القصبات المعدي من الامراض المهمة التي تصيب الجهاز التنفسي لدجاج اللحم مسببا خسائر اقتصادية لصناعة الدواجن. ان الهدف من هذه الدراسة هو لمعرفة التثبيط المناعي للقاح الحي المستخدم ضد مرض التهاب القصبات المعدي على البرامج والطرق اللقاحية المستخدمة ضد مرض النيوكاسل. تم استخدام 125 فرخ لحم بعمر يوم واحد حيث قسمت هذه الافراخ الى خمسة مجاميع متساوية, المجموعة الاولى لقحت بلقاح نيوكاسل والتهاب الشعب الهوائية الحي بطريقة تغطيس الرأس واللقاح المبطل الزيتي (نيوكاسل) بطريقة الحقن تحت الجلد بعمر يوم واحد. المجموعة الثانية لقحت بلقاح نيوكاسل و التهاب الشعب الهوائية الحي بطريقة الحقن بالمجمع واللقاح المبطل الزيتي بطريقة الحقن تحت الجلد بعمر يوم واحد. المجموعة الثالثة لقحت بلقاح نيوكاسل الحي لوحده بطريقة التغطيس و ثم بالحقن تحت الجلد باللقاح الزيتي المبطل بعمر يوم واحد, المجموعة الرابعة لقحت بلقاح نيوكاسل الحي بطريقة الحقن بالمجمع واللقاح المبطل الزيتي بالحقن تحت الجلد بعمر يوم واحد. فيما تركت المجموعة الخامسة بدون تلقيح كمجموعة سيطرة. كل المجاميع المعاملة لقحت بلقاح النيوكاسل الحي بطريقة الرش بعمر 10 ايام. تم قياس التثبيط المناعي للقاح التهاب القصبات المعدي اعتمادا على اختبار اثباط التلازن الدموي واختبار التحدي بالعترة المحلية الضارية واختبار مؤشر جراب فابريشيا وكذلك منسب الطحال. بينت النتائج ان كلا المجموعتين الثالثة والرابعة اظهرت مستوى مناعي اعلى مقارنة بالمجموعة الاولى والثانية. ولم تظهر فروقات معنوية في منسب جراب فابريشيا ومنسب الطحال. اما نتائج اختبار التحدي بالعترة الضارية لفائروس نيوكاسل فكانت نسبة الهلاكات في المجموعة الثالثة 0% والمجموعة الرابعة 10% والمجموعة الاولى 85% والمجموعة الثانية 90%. استنتج من هذه الدراسة ان افضل برنامج لقاحي كان بطريقة التلقيح بتغطيس الرأس بلقاح النيوكاسل لوحده مع التلقيح بلقاح النيوكاسل المبطل الزيتي بعمر يوم واحد والذي كان في المجموعة الثالثة.