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Effect of hyperimmunized egg yolk on maternal immunity of Newcastle disease vaccine in broiler chicks

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Abstract

Hyperimmunized egg yolk (HIY) was produced in two layer hens by four successive immunization with live attenuated Newcastle disease vaccine (NDV) by seven days intervals for each vaccination process. Fifty broiler chicks were used for treatment with hyperimmunized yolk. They were divided into five groups. The first group was treated at 14th days of age by orally and intramuscular injection (five chicken for each route). The second group was boostered with (HIY) after 7 days of first dose at14 days of age. The third group as that of the first group but treated at 21st day of age. The fourth group was treated at 21st days and boostered after 7 days. The fifth group was ten chicks remain without any treatment used as control comparison of all groups. Immune response was measured using HI technique.The results showed that the group of 14th day of age with booster dose gave high antibody titer by intramuscular injection (second group).

Keywords: Newcastle disease; Hyperimmunized yolk; Broiler; HI test; maternal immunity.
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تأثير مح البيض عالي التمنيع على المناعة الامية للقاح مرض نيوكاسل في فروج اللحم

أنوار زكي الزبيدى و مزاحم ياسين العطار

فرع ألاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم تربية اثنين من الدجاج البياض لإنتاج مح عالي التمنيع بإعطائها لقاح نيوكاسل الحي المضعف أربعة جرعات منشطة متتالية يفصل بينها سبعة ايام. استخدم ٥٠ فرخ قسمت إلى خمسة مجاميع، ضمت المجموعة الأولى عشرة أفراخ أعطيت المح عالي التمنيع بعمر ١٤ يوم خمسة أفراخ عن طريق الفم والخمسة الباقية عن طريق الحقن بالعضل أما المجموعة الثانية فقد أعطيت جرعة منشطة من المح بعد الجرعة الأولى بفارق سبعة ايام وبنفس الطريقة المجموعة السابقة، المجموعة الثالثة عوملت نفس معاملة المجموعة الأولى ولكن بعمر ٢١ يوم. اما المجموعة الرابعة فقد عوملت بعمر ٢١ يوم ونشطت بعد سبعة ايام، وقد تركت عشرة أفراخ بدون معاملة كمجموعة سيطرة (المجموعة الخامسة) أظهرت النتائج أن أفضل مجموعة كانت لأفراخ (المجموعة الثانية) بعمر١٤ يوم مع الجرعة المنشطة وعن طريق الحقن بالعضل، وقد درست الاستجابة المناعية باستخدام تقنية تثبيط التلازن الدموي.

Introduction

The immune system of poultry is a complex network of different cell types and soluble factors that give effective response to pathogenic challenges. The function of the immune system is directly associated with poultry health (1). Among the infectious diseases, ND is a deadly viral disease of poultry due to its high contagiousness and rapid spreading character. Vaccination for protecting chicken from ND is routinely practiced throughout the world, but the vaccination is ineffective with the high titer of maternal antibodies (2,3). In birds the maternal antibodies in the egg is manifested by three classes of antibodies in chicken namely IgY, IgA and IgM (4-6), IgY is present in the egg yolk whereas IgA and IgM are present in the egg white as a result of mucosal secretion in the oviduct, the transfer of IgY from the dam to her offspring takes place in a two step process in the first step IgY is taken up into the egg yolk by IgY receptors on the ovarian follicle from the dam's blood through the granulosa cells in the second step IgY is

transferred from the egg yolk to the offspring via the embryonic circulation (4,6-8). This type of immunoglobulin is act as passive immunity because these antibodies are derived from the dam and protect the offspring from various infectious disease (9). Chicken can provide a convenient and economic source of immunoglobulin in their egg yolk, the efficiency of egg yolk immunoglobulin (IgY) has been assessed for therapeutic application by passive immunization therapy for prevention or control of different infections (10) Many reports were studied the prophylactic effect of egg yolk IgY and its success in the prevention of different pathogens as *Helicobacter pylori (11),* enterotoxicogenic, *E. Coli, Streptococcus mutans, Salmonellosis, Campylobacterosis*, mixed bacterial infections, *Staphylococcus* with *Pseudomonas* and viral diseases as Infectious bursal disease, Newcastle disease, Rota virus, (12-16).

This paper describes the production of hyperimmunized egg yolk (HIY) in layer chicken and the purification of egg yolk in order to study its effect on maternal derived antibody titers of NDV.

Materials and methods

Production of hyperimmunized yolk

Two layer chickens (22) weeks old were vaccinated with one vaccinal lasota strain of NDV (Lohmann company) dose of (10^7 EID_{50}/ 0.1ml) orally for four times with seven days interval between each vaccination.

Collection of egg samples

Eggs were collected once before ND vaccination and other four groups of eggs were collected after one week after each immunization then were stored at 4°C until further use.

Separation of egg yolk

Egg yolk was taken out of the egg shell, placed in a sterile container and mixed with the same volume of sterile phosphate buffer saline (PBS) with shaking. The emulsion was centrifuged at 1500 rpm/ 20min, The mixture was separated into 2 layers, the clear supernatant fluid containing the IgY was collected and stored at -20°C until analysis (17,18).

Experimental chicks

Fifty-one-day- broiler chicks were housed in animal house of veterinary medicine college and kept for ad libitum containing no antibiotics. All birds were not vaccinated,ten were chicks used as control group without any treatment and 40 chicks were divided into four groups

First group: Ten chicks on 14th days old were given 1ml of IgY containing fluid, by oral and I/M injection routes as a single dose, five chicks for each route of administration).

Second group: Ten chicks on 14th days old were given 1ml of IgY containing fluid followed by boostering dose after one week at the routes and number of birds.

Third group: Ten chicks on 21st days old were divided into two subgroups 5 chick were given IgY containing fluid 1ml by oral and I/M injection as single dose.

Fourth group: Ten chicks on 21st days old were given 1 ml of IgY containing fluid with boostering dose after one week from the first dose by oral and 1/M routes (5birds for each route).

Fifth group: Ten chicks remain without any treatment and regarded as negative control.

Collection of serum samples

Blood samples were collected from all experimental groups by heart puncture then sera were separated by centrifugation at 2000rpm for ten minutes and were stored at -20°C until further use.

HI test

Antibody titer against Newcastle disease virus (NDV) in serum and yolk was determined by (HI) technique depending on (4HA) units (17).

Statistical analysis

Data of all experiments were expressed as mean ± SE data were compared by one way and tow way analysis of variance. Significant differenced determined by holm-sidak and Duncan's multiple range test. All statistical analysis performed by sigma stat (Jandel scientific software v3.1) ($P<0.05$) was considered as statistically significant.

Results

Newcastle disease antibody titres in laying hens

The results of the present study revealed that the antibody titre of hyperimmunized yolk were different significantly ($P<0.05$) in the collected eggs. The higher HI titer against NDV was 4.625 when layer chickens were vaccinated with live attenuated Newcastle disease vaccine with three booster doses, (Table 1).

Newcastle disease antibody titre in broiler chicks

The serum antibody titres of control group (fifth group) show the high maternal derived antibody titer through the two weeks only (5.250, 4.375, 3.625) then decline gradually to reach 0.833 at five weeks of age, (Table 2).

Table (1): Mean± SE of HI titre in egg yolk after successive NDVvaccination of layer hens.

Numbers of vaccination to layer chickens	No.of examined eggs	Mean ± SE HI titre of egg yolk
One vaccine only	5	2.800 ± 0.200 A*
vaccine+one booster dose	8	3.00± 0.267 A
vaccine +two booster doses	8	4.125± 0.295 B
vaccine +three booster doses	8	4.625 ± 0.183B
Control	8	2.375 ± 0.263 A

*A-B values within a column following by different letters are significantly different (P<0.05).

Table 2: Mean± SE HI maternal antibody titres of control group (fifth group) chicks.

Age of birds	Mean ± SE HI serum titre
One Day	5.250 ± 1.282 a*
7 Days	4.375 ± 0.744 ab
14 Days	3.625 ± 1.88 bc
21 Days	3.00 ± 0.926 cd
28 Days	2.2 ± 0.447 de
35 Days	0.833 ± 0.753 e

*Different letters in the column revealed significant differentces (P<0.05).

The results of antibody titres of experimentaly treated chicks with hyperimmunized yolk show significantly higher antibody titres with I/M injection compared with those treated with oral route in all groups. The higher titre was 5.8 recorded in the second group (chicks treated in two weeks with booster dose) and also give higher antibody titres of 4.4 by oral route. These titres were higher than those reported in the remaining groups (3 and 4) and in both oral and I/M injection routes (Table 3).

Discussion

The present study show that antibodies as IgY transferred to egg and then given to the newly hatched broiler chicks (4) the hyperimmunized yolk of the layer chicken that vaccinated with live attenuated Newcastle disease vaccine show high yolk antibody titre that increased with repeated immunization of layer hens and this result were coencides with the results of (12,14,17) who revealed that repeated immunization of layer hens with live attenuated vaccine give good yolk antibody titer. The other fact that was reaffirmed in the present study the chicken egg yolk was recognized as the antibody source and the important of using egg yolk immunoglobulin IgY has been assessed for therapeutic application by passive immunization when the chicks treated with hyperimmunized yolk, the higher ratios were seen in 2nd group were treated in two week of age with yolk and boostering after one week with it and this agreed with (10) who revealed that multiple treatment with egg yolk increased the antibody titer and also (9) who consider egg yolk containing high level of antibodies that give protection of 80% of birds against NDV and this fact can be easily applicated in the other infectious diseases of poultry. The current study also revealed that intramuscular injection route is the best method for immunization this result were in line with that of (9,18-21) this could be explained by that oral administrated of egg yolk may be affected by acid denaturation in the proventiculus and gizzard and by proteases, however a fraction of the administrated dose retains some immunological activity against gastrointestinal tract.

In conclusion IgY administration by I/M give great future opportunities for prophylactic strategies to increase and maintain protective level of immunity against NDV and if this approach works well it may help to justify commercial application of these antibodies.

Table 3: Mean ± SE serum antibody titres of treated chicks with IgY containing fluid in different groups.

Type of group	Mean ± SE HI serum antibody titre		
	Oral	1/M injection	Fifth group (control)
First	3.8 ± A*	4.60 ± A	3.625 ± A
	0.270 a**	0.270 b	1.88 a
Second	4.4 ± A	5.8 ± B	3.00 ± A
	0.270 a	0.270 b	0.926 c
Third	2.2 ± B	3.8 ± C	3.00 ± A
	0.270 a	0.270 b	0.926 b
Fourth	1.00 ± C	2.40 ± D	2.2 ± B
	0.246 a	0.270 b	0.447 b

*A-D: values within a column following different letters are significantly different (P<0.05).
**a-c: values within a raw following different letters are significantly different (P<0.05).

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