University of Thi-Qar Journal Of Science (UTsci)
Website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq
Volume 6, Number 2, June 2017

Improvement of Newcastle disease virus vaccine by using gold nanoparticles and some natural food additives

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Abstract

Newcastle is a disease consider is one of important poultry infectious diseases, it cause highly significance economic loss for the poultry industry. 120 one day age chicks is divided into sexgroups (A,B,C,D,E,k) two first group injected with Newcastle vaccine mixed with gold nanoparticles in different concentrations, third group vaccinated then fed Cinnamon powder mixed with bird feed, forth group Nigella stava seeds mixed with birds feed, fifth group Cinnamon oil mixed with vaccine and the last group injected with vaccine only which consider as control group.
Antibodies against Newcastle vaccine and interferon gamma titer are estimated in a different period after vaccination 14, 21, 28 and 35 days by ELISA method. Highly antibodies titer were found in third group after 35 days (0.60 ± 0.07) while the two first groups were given significant results elevated of antibodies during all periods of experiment compare to other groups (0.54).Results showed that there were a significance difference between the groups (A, B, C, D, and E) and control group (K).The study revealed that highest IFN level titer was in group (B) which was (1.91), followed by groups (E, A, K). At 5 weeks old chicks , the highest level of IFN was in group (A)(gold nanoparticles-vaccine group 50:50) (1.91) , followed in group (E , B, C) was (1.64 , 1.62 , 1.61).Significant differences($p< 0.05$) were found between different groups during experiment time.

Key words: Newcastle, gold nanoparticles, poultry, vaccine

تحسين الاستجابة المناعية للقاح مرض النيوكاسل باستخدام ماده الذهب النانوية وبعض محفزات المناعة الطبيعية

الخلاصة

يعتبر مرض النيوكاسل من الامراض المهمه التي تسبب خسائر اقتصادية لصناعة الدواجن على نطاق واسع جداً. شملت الدراسة 120 فرخ دجاج بعمر يوم واحد, قسمت الى ستة مجاميع كل مجموعه 20 طير حقنت المجموعه (ا, ب) بلقاح نيوكاسل ممزوج مع مادة نانوالذهب بتركيزين مختلفين وحقن تحت الجلد, اعطيت المجموعة (ج) مسحوق القرفة مع العلف لمدة 35 يوم كذلك المجموعة(د) حيث اعطيت مجروش الحبة السوداء مع العلف , اعطيت المجموعة الخامسة(ذ) مزيج اللقاح مع زيت القرفة بنفس الكميه تحت الجلد واعطيت المجموعة السادسة (ر) مادة اللقاح فقط واعتبرت كمجموعة

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سيطرة. تم قياس تركيز الاجسام المضادة والانترفيرون كاما ولفترات مختلفة 14, 21, 28, 35 يوم بعد اعطاء اللقاح بطريقة الاليزا. اظهرت النتائج ان افضل مستوى للاجاسم المضادة للفايروس في المجموعة الثالثة حيث كانت النتائج (0.07 ± 0.60) بعد 35 يوم من بداية التجربة. بينما اعطت المجموعة الاولى والثانية نتائج بفارق معنوي عن مجموعة السيطرة خلال فترة التجربة (0.54) كذلك اهرت المجاميع الخمسة فرقا معنويا على مستوى احتمالية اقل من 0.05% مقارنةً بمجموعة السيطرة. بالنسبة لتركيز النترفيرون كاما فقد اعطت المجموعة الثانية (ب) اعلى تركيز حيث بلغت (1.91) بعدها المجموعة الاولى (ا) و المجموعة الخامسة (ر) بعد 14 يوم من بدء التجربة. بينما اعطت المجموعة (ا) اعلى تركيز بعد 35 يوم من التجربية حيث كان التركيز (1.91). واظهرت المجاميع (الخامسة , الثانية والثالثة) النتائج التالية .(1.61 , 1.62 , 1.64) بالتعاقب واظهرت فرقاً معنوياً على مستولى احتمالية (p< 0.05).

Introduction

Newcastle disease (ND) is an infectious disease affecting domestic and wild birds of any age. It is probably the most serious disease of chickens throughout the world, which is a major economic concern. (Alexander, 2000 ;Al-Mola and Sehry, 2014)
A highly contagious and sever form of the disease called exotic Newcastle disease (END) is highly pathogenic so bird die suddenly without showing any sign of disease , in susceptible chicken , mortality may exceed 95% (Aldous and Alexander ,2001 ; Mahdi and Naser, 2014)
The great impact of ND may be on villages in the developing countries throughout Asia , Africa , central America and some parts of the south America, the village chicken is an extremely important asset representing a significant source of protein in the form of eggs and meat . However, ND is frequently responsible for devastating losses in village poultry. A large majority of countries rearing poultry commercially rely on vaccination to control ND, but ND nevertheless represents a major limiting factor for increasing poultry production in many countries. (Alexander, 2000)
ND is prevent and controlled by vaccination or by quarantine and slaughter of disease flocks in confirmed outbreaks (Alexander and Senna, 2008 b)
NDV strains used in conventional commercial live and inactivcatedvaccines .live vaccine fall into two groups: lentogenic and mesogenic vaccine (OIE , 2012)
Most commercially available ND vaccines are inactivated whole-virus preparations containing oil emulsions as an adjuvant to improve their efficacy .However , the vaccines have been reported to induce poor immune response. Therefore , there is need to improve currently available ND vaccines to effectively protect animals from ND infection (Xiaeet al. ; 2009)
When devising a vaccination programme, consideration should be given to the type of vaccine used, the immune and disease status of the birds to be vaccinated and the level of protection required in relation to any possibility to infection with field virus under local conditions (OIE , 2013)
However, with use different vaccine programs but , ND still threat to the poultry industry in most countries of the world , including Iraq , where it appeared many emerged of ND because virulent strain causing the disease , type of strain vaccine , method of administration of the vaccine, as well as , a failed to follow good program for vaccination lead to disease control and lack of commitment farmers immunization programs and their dependence on the personal endeavor of vaccination programs (Alexander,2000 ; Alexander,2003)

Materials and Methods

A total of 120 one day age the chicks (Ross) were randomly divided into 6 groups each one consist of 20 birds, each one of groups was the injected with Newcastle vaccine (ND +Flu)Lasota killed strain,(0.5 cc 3/bird) mixed with different materials to improve immune responses as following: experiment extend from 15 November to30 December 2015 in Thi-qar province in Iraq.

1-Group A: The birds were immunized by a single neck subcutaneous injection (1cc) of mixture from (50:50) Newcastle vaccine and citrate Nanoxact Goldnanparticles (Compamix. USA). Mixture homogenous well by shaker under cold conditions.

2- Group B: The birds were immunized by a single s/c injection (0.75 cc) of mixture of (25-75) Citrate Nanoxact Gold nanoparticles and Newcastle vaccine respectively.

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3-Group C: The birds vaccinated with ND vaccine by single s/c injection (0.5 cc) , and then supplemented of Cinnamon powder 1
00 gm/ 5 kg of chicks feed.

4-Group D: The birds vaccinated with ND vaccine by single subcutaneously injection (0.5 cc) , and then supplemented Nigella Sativa seeds 125 gr/ 5 kg (0.025 gr) mixed well with chicks feed.

5- Group E: The birds immunized by a single subcutaneously injection (1cc) of mixture from (25:75) of Cinnamon oil and Newcastle vaccine.

6-Group K : The bird vaccinated with ND vaccine by single subcutaneously injection of Newcastle vaccine without supplements (0.5 cc) as controlgroup .

Antibodies titers and interferon gamma estimated by ELISA methods, materials used supplied by (Biomysources, USA). The Periods for the detection antibodies were 2 weeks, 3 weeks, 4weeks and 5 weeks after vaccination. Blood were collected from the wing vein by sterile syringe about 2 ml of blood the collected leave in the room temperature for the coagulate then serum used for detected antibodies and interferon gamma against Newcastle virus as a manufacture procedure of ELISA kit. Micro titer plates read by ELISA reader (BiokitElx 800 in USA)
Statistical analysis was done by using spss one way anova.

Results

Results of antibodies titers of different groups and periods revealed in table (1)

Table 1: Antibody titer of ND vaccine during the experiment time by ELISA method

Group	2week	3week	4week	5week	L.S.D
A	0.46 ± 0.21 ^{a}	0.39 ± 0.06^{a}	0.41 ± 0.04^{a}	0.54±0.06^{a}	0.17
B	0.34 ± 0.01 ^{b}	0.42 ± 0.08^{ab}	0.52 ± 0.20^{a}	0.54 ± 0.02 ^{a}	0.166
C	0.40 ± 0.06 ^{b}	0.42 ± 0.01 ^{b}	0.40 ± 0.07 ^{b}	0.60 ± 0.07 ^{a}	0.127
D	0.38 ± 0.04 ^{a}	0.41± 0.06 ^{a}	0.42 ± 0.07 ^{a}	0.35± 0.08 ^{a}	0.107
E	0.37± 0.02 Bc	0.36 ± 0.03c	0.46 ± 0.03 ^{a}	0.40 ± 0.03 ^{b}	0.04
K	0.37 ± 0.03^{a}	0.36 ± 0.09^{ab}	0.29 ± 0.01^{b}	0.29 ± 0.02^{b}	0.08

After 2 weeks of vaccination the highest antibody titers in group (A) value were (0.463), followed by group (C) value was (0.407).
Whereas, the lowest level of antibody titer was in group (B) value was (0.345). However, group (D, K, E) gave medium level of antibodies titers which were (0.391, 0.378 , 0.372) respectively .Significant differences ($p < 0.05$) found between group (A) and (B) , (C) and (D) and between (E) , (K) There is no significant differences ($p > 0.05$) between groups (A), (D) ,(k) also not found between groups (B), (C) ,(E).
At 21 days old chicks, the highest level of antibody titer was given by group (B ,C) which was (0.427 , 0.420) . followed by group D which was nearly the same level titer (0.416) .whereas , the lowest level of antibody titer was given by group (K,E) which was (0.36 , 0.363) respectively .Significant differences were found among different groups ($p < 0.05$) between (A) ,(C) and between (A ,B,D ,K), (E) .There is no significant differences ($p >0.05$) between groups (A) ,(B) ,(D) , (K).

However, some groups like (B, C, D) reflected increase of immune response level in comparison with that of 14 days old.
At 28 days old chicks, the highest level of antibody titer was given by group (B) was (0.530), followed by group (E) which was (0. 437. However, groups (D, A,
C) Gave medium level of antibody titer which was (o.425, 0.413, 0.403) respectively .The lowest level of antibody titer was given by group (K) which given (0.293). Significant differences ($p < 0.05$) foundbetween (A),(C) and between(B) , (K).There are no significant differences ($p> 0.05$) among groups (A), (B), (D), (E) Also not found between groups (C), (K) .However, some groups (A , B, D, E) reflected increase of immune response level comparison with that of 21 days old .
At 35 days old chicks, the highest level of antibody titer was given by group (C) Which was (0.606) , followed by groups (A, B) which was (0.552 , 0.543) respectively . The lowest level of antibody was in group (K) (0.294).

There is significant differences ($p < 0.05$) were found between (A, B,C ,D) comparison with (E , K) and no significant differences ($p > 0.05$) between groups (A,B, C, D) . However, some groups (A,B , C) reflected increase of immune response level comparison with that of 28 days old.

Table 2 result of interferon gamma level during experiment time by ELISA test

groups	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	LDS
A	1.81 ± 0.16^{ab}	1.68 ± 0.06^{b}	1.73 ± 0.16 ^{ab}	1.91 ± 0.06 ^{a}	0.192
B	1.91 ± 0.35 ^{a}	1.64 ± 0.6 ^{a}	1.75 ± 0.13 ^{a}	1.62 ± 0.18 ^{a}	0.325
C	1.69 ± 0.19^{b}	1.62 ± 0.05^{b}	1.96 ± 0.18^{a}	1.61 ± 0.10 ^{b}	0.225
D	1.43 ± 0.72^{a}	1.51 ± 0.27^{a}	1.55± 0.04^{a}	1.52 ± 0.21^{a}	0.607
E	1.85 ± 0.04 ^{a}	1.63 ± 0.06 ^{b}	1.52 ± 0.14^{b}	1.64 ± 0.02 ^{b}	0.214
F	1.80 ± 0.15 ^{a}	1.65 ± 0.05^{ab}	1.57 ± 0.04^{b}	1.41 ± 0.27	0.244

Statistical analysis in table (2) , showed in age 2 week old chicks , the highest IFN level titer was in group (B) was (1.91) , followed by groups (E, A, K) which nearly to the same level was (1.85 , 1.81 , 1.80) respectively .

The lowest IFN titer in group (D) was (1.43). Significant differences ($p< 0.05$) were found between different groups .

At 3 week old chicks , the highest level of IFN was in group (A) was (1.68) , followed in group (K , B ,E , C) was (1.65 , 1.64 , 1.63 , 1.62) respectively , and the lowest level of IFN was in group (D) (1.51) .
Significant differences ($p< 0.05$) were found between different groups. However, reflected decrease of IFN level comparison with that of 2week.

At 4 week old chicks , the highest level of IFN was in group (C) (1.96) , followed in groups (B, A) was (1.75 , 1.73) , and lowest level of IFN in group(E) was (1.52) .
Significant differences werefound between the groups ($p< 0.05$) .However, some group (A, B , C, D) reflected increase of IFN level comparison with that of 3 week.

At 5 weeks old chicks , the highest level of IFN was in group (A) (1.91) , followed in group (E , B, C) was (1.64 , 1.62 , 1.61) which nearly to the same level. The lowest level of IFN-Ɣ in group (K) was (1.41). Significant differences ($p< 0.05$) was found between different groups. some groups (A,E) reflected increase the level of IFN and other groups (B , C, D, K) reflected decrease of level of IFN comparison with that of 4 weeks.

Discussion

The level of the antibodies are represent the humeral immune the response, at the14 days – old chicks .There was the clear the different of the antibodies titer the among all vaccinated groups at this period , this may be due to the different level of the interaction the between the maternal the antibodies and the liberated virus from the vaccine . This finding in the consistent with Awanget al., (2000) who reported that the vaccinated the birds the showing individual the variation in the production of the antibodies at the 14 days old as estimated by ELISA.
On the other hand, the results is agreement with the previous study of Al- Baroudi, (2001) who he recorded the different of immune response the antibodies and mentioned that this variation might be due to the presence of the variable passive immune in the chicks, or to the varying the degree of susceptibility of the immune mechanism to the antigen as were also the suggested by.
Bozarhmehrifardl and Mayahi(2006) , suggested that these variation in the Antibodies titer might be due to the genetic incapability of the some birds to the produce any reaction to NDV and the genetic the constitution of the birds may have a the significant the effect on the response to the vaccination.
Furthermore, (Koch et al. 2000) discussed that the variation in the antibody the titer after vaccination may be due to the variation in the vaccine strains. This finding theindicate that a period of the 2 week is not sufficient to thereflect a differences in the immunological the response among the different the groups of birds.
At 14 days of the age , there is the significant differences the recorded by ELISA test ($p < 0.05$).Ghummanet al., (2000) noted that ELISA test was the more the sensitive than other serological test in detection of the antibody in the chicks , and it detect all functional the type antibodies , in addition , ELISA test able to the detect the antibodies activity against NDV in field the sample and it the measures antibodies with a wide the antigenic the spectrum.
Later on at 21 days old chicks , there is variation in antibody titer was increase in more than 2 week , also there was a significant differences ($p < 0.05$).
This increased of the titer in the former groups was due to the slow the liberation of the viral antigen , so

longer time need for the neutralization of the maternal antibody and give the more chance for the continuous the stimulation of the immune response.

This finding in agreement with the finding of (Russell, 2001) , who discussed all groups at 21 days the reflected a titer the more than 14 days that due to the liberated viral antigen was the neutralize all the maternally antibodies and the effect of the adjuvant the vaccine that appear more clear in the later period which was gradually increased.

However , by 21` of age , the mean antibody titer was the statistically higher than those of day 14 of age , indicating the drop in the maternal immunity to low level between 2 and 3 week of age that allowing for vaccine virus the replication. At this age, the significant the differences was the detected among the different groups in the antibody titer.

At 28 days –old chicks, the titer of antibody in all vaccinated groups is higher comparison with level of 21 day old. At this time, the effect of maternal immunity was the diminished gradually and its effect the decrease with the time progress.

The inactivated the vaccine dispenses antigen the slowly and the providing a progressive the stimulation of the immunity while the acquired maternal immunity declines whereas the live vaccine induces the immediate the local tissue the immunity.

At 35 day – old chicks, the titer of the antibody highly increase comparison with 28 days – old chicks.

Generally speaking , the titer of the immune response start to the increase from 21 days the upward in groups which were the vaccinated at 7 days old which may be the due to the both a continual the maturation of the immune the system is not complete the until least 1 week of the age , thus the ability to the response to a particular the antigen might be the delayed and the declination of the maternal antibodies that are the transferred to the progeny and the prevent a full response to the vaccination.

This finding in agreement with the previous work of Aggar , who found that the vaccination of chicks at the 7 days –old gave higher level of the antibody titer and better protection rate the against challenge dose with virulent NDV . This finding the encourage us to the vaccine chicks at the 7 days –old rather than 1 day-old for the better the immune the response and the protection.

Darminto and Ronharjo, (2000) found that the inactivated vaccine prepared from a velogenic local strain of NDV gave higher immune response and mentioned that this inactivated vaccine highly immunogenic compared to that of live vaccine.

5-2- Result of Interferon Gamma

Interferon gamma (IFN-γ), an immunoregulatory cytokine, is known to control many microbial infections. Chicken interferon gamma ((chIFN-γ) was stimulated chicken embryo fibroblasts (CEFs) inbnhibited the replication of viral RNA (vRNA) and showed a mild decrease in the infectious virus load released in the culture medium. (Su Yuk et al., 2016).Gamma interferon (IFN-γ) has been shown to be an important component of the host protective Cell-mediated immunity (CMI) (Choi et al., 2001; Lillehoj and Choi, 2000) . Thus, IFN-γ production is used as an indicator of CMI in various avian disease models (Kaiser et al., 2000 ; Lambrechtet al., 2004).

Chicken interferon γ (ChIFN-γ) production as an indicator of actively acquired immunity to NDV. The CMI response measured by the ChIFN-γ ELISA carries great potential for representing the role of CMI in protection against avian ND as well as facilitating the study of the role of this cytokine in various immune mechanisms in the chicken. (Khalifeh et al., 2009). There are several methods available for the measurement of interferon gamma or T-cell responses in chickens. Production of IFNγ can be evaluated by enzyme-linked immunosorbent ELISA . (Rauw et al., 2009). The result showed after 1 week from vaccination (14 days –old age) had a significant increase in ChIFN-Ɣ production .

This high background level of ChIFN-γ production masked the effect of NDV antigen recall stimulation in these groups, which in the vaccinated . This is similar to the effecton protective CMI response after NDV vaccination in chickens, which resulted in an increase in the peripheral lymphoproliferation activity (Munir et al., 2009 ; Khalifa et al., 2009).

Because IFN-γ is the signature cytokine for T helper 1 cells, it is important to assess the level of this cytokine production and use it as an indication of the CMI response (Sharma, 2000).

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