SHORT ARTICLE / INVESTIGACIÓN

Evaluation of humoral immunity by IgG measurement in broilers vaccinated with life and killed Newcastle vaccine

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Abstract: Current research objectives evaluated the humeral immunity induced by two different vaccination methods and estimated the efficiency of the commercially available Newcastle disease (ND) vaccine. A total number of 90-day-old unvaccinated chicks were divided equally into 3 groups; each group was vaccinated with different vaccination methods, while group 3 was the unvaccinated control group. Serum was collected from all groups, and five chickens from each group were slaughtered. ELISA measured the levels of IgG; there also were higher in the vaccinated groups (G1, G2) compared with the unvaccinated group. Group 2 also had the best IgG; The higher values of lymphoid organs (Bursa of Fabricius, thymus and spleen) indices were in. While the vaccinated groups were compared to non-vaccinated groups, between vaccinated groups, there was no significant difference ($P < 0.05$). The best-proven vaccine program in group 2(primary vaccine at 7th day of old by the intraocular vaccine (live vaccine), then boost potion dose at 21st day of old by drinking water vaccine(live vaccine).

Key words: Chicks, humeral immunity, vaccine.

Introduction

Newcastle Disease is a sensitive and highly fatal; contagious viral disease combined with neurological and respiratory signs that affect a widespread domestic and wild bird species throughout the five continents of the world1. Created by (NDV); an avian paramyxovirus type[1] ND By pathotyping, NDV strains were classified into the highly pathogenic depending on the virulence in poultries: ketogenic, mesogenic and velogenice[2,3].

The clinical form affecting the neurological, gastrointestinal, reproductive, and respiratory systems is often noted in naïve, unvaccinated, or poorly vaccinated birds. Clinical signs vary depending on the species of bird, the strain and challenge dose of the virus, and the immunity of the host[4]. The most common symptoms are respirational signs such as coughing, panting, sneezing and rales. Additional signs include falling wings, tedious legs, enlargement of tissues around the neck and eyes, twisted neck, rotating and interruption of egg creation.

Vaccination and appropriate hygiene measures are the most effective way to control and prevent ND. ND immunization plans include inactivated and attenuated vaccines to improve protection from infectious diseases[5]. Killed vaccines include viruses without pathogenic properties. It is inactivated through physical and chemical ways; it provides very high levels of antibodies against NDV and produces good protection against the virulent virus[6]. It was applied by injection (S/C, IM) and has provided excellent antibody levels. In contrast, the live vaccine was applied by eye drops or drinking water technique to stimulate local defensive immunity managed by (Ig)A antibodies[7].

N D V infection stimulates innate and humeral immunity; the innate immune response is an immediate reaction to control and inhibits the growth of viruses, spreading and aiding in developing pathogen-specific protection through the adaptive immune response[8,9].

Humeral immunity eliminates extracellular pathogens (NDV) in body fluids and produces antibodies secreted by plasma cells after activation from B lymphocytes called IgG. The IgG is the most critical protective in avian immunity and can be measured in some cases in serum. The antibody titer levels still peak for 2-4 weeks from infection or vaccination[10].

In Iraq, numerous live vaccines with lentogenic strains of NDV, such as LaSota, are approved by several traders. However, these vaccines' efficiency in climatical state, spreading and transport are not inspected correctly and wisely by the trader or handler[11].

Materials and methods

Chicks

A total number of unvaccinated birds, 90 on 1st day of age, were taken from a local hatchery. Whole birds were adapted in their cages and provided with feeding and water during the experiment period; before the applied program, the birds were subdivided into 3 groups, each group included 30 birds, and a third group was considered the control group.

N D vaccines

This research used two types of vaccines; live vaccine Izovac NDV LASOTA and killed vaccine Nobilis ND BROILER.

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Experimental design

The experimentation persisted for 5 weeks, as The vaccination regimen (designed on the local recommended program, as shown in Table 1.

Sample collection and sampling schedule

Two ml of blood was collected aseptically from each bird's wing or jugular vein and allowed to clot, and serum was separated using a bench centrifuge at 1500 rpm for 10 min. Then sera were separated and stored at -20 o C until the serological tests were carried out. Pre-vaccination sera were randomly collected from 25% of the birds used for the study on the seventh day following hatch to evaluate their maternal immune status. Six chickens were sampled randomly from each group.

Measurement of antibodies (IgG) against NDV by commercial ELISA kit

Used commercial ELISA kit (Newcastle Disease Virus Antibodies ELISA Kit, Catalog No: E-AD-E013). ELISA was performed as per the manufacturer's instructions; This ELISA kit applies to the in vitro quantitative determination antibodies against NDV

Microplate hemagglutination inhibition (H I) test

Microplate H I applied to limit the ab level of the obtained sera as of the bird in the three groups. The IgG level of the NDV in the serum was estimated by H I and cross HI tests in U-bottom microtiter plates using constant 8 H A units of LaSota strain as antigen with two-fold serum dilutions (β method). HI, titers equal to or greater than 1/ 16 (24) were considered positive as the World Organization for Animal Health recommended.

Lymphoid organ indexes

In the last week of the experiment (35 days), each group was weighed individually; 5 birds of each group were sacrificed .than a thorough visual evaluation, the thymus, bursa of Fabricius and spleen were immediately removed, dry and individually weighed. Advanced significant lymphoid organ weight variation was assessed, and their directories were planned[12].

Statistical analysist

The data collected in this research in different groups were statistically inspected via analysis methods of alteration (ANOVA). P<0.05 was measured as statistically significant.

Results

Hemagglutination inhibition (HI) test

Hemagglutination was 1:128 at the last dilution of ag (antigen); the antigen titer(8 HUA) was used. The antibody response of the chickens to vaccination schemes was assumed in Table (2); The mean pre-immunization HI antibody titer was found to be 4.6 at age 7 days, then declined to 2.6 and 0 at age 21 and 35 days, respectively.

Post-vaccination HI geometric mean titer (GMT) values were determined, and it was detected that on day 21 of age (14 days post-vaccination), all the birds in Group 1,2 seroconverted with a statistically significant increase ($p < 0.05$). Serum HI antibody titers that were protective. However, the birds in group 1, which had higher HI titers.

Titer of IgG concentration in serum

Serum antibody titers against NDV antigen at the first, third, and fifth week of age in all groups are obtainable in Table 3. The antibody titers against NDV was exhibited as a percentage of inhibition (IP), and the results show that maternal immunity dropped to a non-protective level at 7 days of age of chicks).

The immunized groups gave good IP comparison with the control or non-immunized groups at 14 days post-vaccination and post-booster doses at 21 days and 35 days of chicken age, respectively, as shown in Table 3.

The immunization program, which was practical in group 2, gave the highest IP, reaching 71 at 14 days post-vaccination (21 days of age) and 88 at 14 days post-booster vaccination dose (35 days of age). The result of the other group (group 1) was 58 and 73 at 21 days and 35 days of age, respectively.

Lymphoid organs indices

The higher values seem in the immunized group compared with non-immunized groups at day 35 (4th week). In contrast, between immunized groups, there was no significant difference ($P < 0.05$) in lymphoid organ indices.

Vaccination day	G1	G2	G3 (control group)
7th day	ND killed vaccine (S/C)	ND live vaccine (I/O)	Unvaccinated
21st day	ND live vaccine (D/W)	ND live vaccine (D/W)	unvaccinated

S/C = Subcutaneously, I/O= Intra Ocular, D/W= Drinking Water

Table 1. The experimental design.

Group	NDV antibody titers		
	At 7 days (maternal immunity)	At 21 days	At 35 days
G1	4.8±1.78	57.6±14.31b	230.40±57.24bc
G2		179.20 ±70.10 a	358.40±140.21a
G3		2.8±1.09 c	0±0.00c

Table 2. The GMT of antibody titers against NDV in experimental groups measured by HI test.

P≤0.05, a small letter describes the significant difference between groups.

Group	NDV antibody titers (percentage of inhibition)		
	At 7 days (maternal immunity)	At 21 days	At 35 days
G1	18±2.54	58±2.54b	73±1.97c
G2		70.8±1.41a	88.8±2.26a
G3		9.2±1.06c	0±0.00d

Table 3. The mean value of antibody titers (percentage of inhibition) against NDV in experimental groups measured by ELISA test.

Group	Indices of Lymphoid Organs at day 35 of age		
	Bursa of Fabricius	Spleen	Thymus
G1	0.095±0.02	0.093±0.005a	0.171±0.01
G2	0.084±0.01	0.093±0.01a	0.194±0.02
G3	0.076±0.02	0.045±0.01b	0.142±0.02

Table 4. Lymphoid organs indices of experimental groups.

Discussion

In the current research, the primary vaccination was approved on time as soon as the parental antibody titer was (GMT 4.8). The results showed that the maternal antibody titer on the 7th day of old was 4.8; the decline in antibody titer was recorded at the 3rd week of age and reached to untraceable at the 5th week of age measured by using hemagglutination inhibition test investigative test for N D . Early protection was necessary with traditional ND vaccines managed at an early period of age to avoid any possibility of infection.

We assessed whether the presently used vaccines under different vaccination schedules could induce effective immunity in chickens. The comparative efficacy of commercially available vaccines has been a challenge for scientists.

The results of the research showed that the antibodies titer was significantly different between treated groups, and the best-increased antibody levels were in group 2which used a live vaccine by eye drop at the 7th day of age and by drinking water at the 21st of age. Also, on days 21st and 35th of age, there were significant differences between the vaccinated and control groups ($p \leq 0.05$).

In the control group, in which any N D vaccine was not used, antibody titers were decreased, and the unvaccinated chickens were susceptible to disease.

Researchers indicated that although immunization mainly provides good protection against disease and mortality, it may not provide sufficient protection against virus transmission to prevent or halt epidemics of Newcastle Disease.

Generally, analyses indicate that a high fraction of birds (>85%) needs to have a high antibody titer after vaccination to ensure that no epidemic spread is possible in vaccinated populations[13,14].

This result is also informed in research that intraocular administration produces higher protection for all vaccines than drinking water vaccine[15].

Parry[16] also found that live ND vaccines administered by eye drops or orally induce protective mucosal immunity mediated by IgA antibodies. It is speculated that the results obtained in our study indicate that the high antibody titer in group 2 on day 35 is due to the booster vaccination (day 21) with the La Sota virus, which replicated quickly in the mucosal membrane, inducing local immunity.

Conclusions

Current studies show that the vaccine given by the eye drop method is better than the drinking water method, and the live vaccine is better than the killed vaccine. The immunity detected in immunized birds indicated that the vaccines were effective. The antibody titer built-up in the tested birds was significant. The two-fold increase in humoral responses of the birds following the 'primer dose' and 'booster dose' was commeasurable with the efficacy of the imported vaccines. This is in tandem with the work of Abbas and Alexander[17,18], who, in their various report, documented on NDV dose-response relationship 63 among the virus content, serological response and clinical protection.

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