Basrah Journal of Veterinary Research,Vol.19, No.3, 2020. Proceeding of the 17th International Conference. College of Veterinary Medicine. University of Basrah. Iraq.

ASSESSMENT OF IMMUNITY INDUCED BY NEWCASTLE DISEASE VIRUS VACCINES AND DETERMINE THE BEST VACCINATION PROGRAM IN BROILER CHICKEN

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Key words: chicks, vaccination, hem agglutination, Newcastle diseases.

ABSTRACT

The objectives of current research were evaluate the efficacy of commercially available Newcastle disease vaccine and determine the best vaccination program. A total number of 150 one-day-old unvaccinated chicks were divided equally into 5 groups. One vaccination program were used for each group which differ from each other while group 5 was unvaccinated control group. Serum were collected from all groups and five chickens from each group were sacrificed. Afterward immunization HI geometric mean titer (GMT) rates were observed that both seroconverted birds in Group 1 to Group 4 have risen statistically significantly, with statistical significant changes. ($p < 0.05$). However, the birds in group 4 which had the best HI titers (147). The levels of ChIFN-γ was measured by ELISA, there were also higher in the vaccinated groups (group 1, 2, 3 and 4) than in the non-vaccinated group. Group 4 also had the best ChIFN-γ level. The higher values of lymphoid organs (spleen, Bursa of Fabricius and thymus) indices were in vaccinated groups are compared to non-vaccinated groups, while between vaccinated groups there was no significant different ($P < 0.05$). Commercial ND vaccines are effective and vaccination scheme of group 4 (live ND vaccine at 7th day of age by eye drop as primary vaccine followed by live ND vaccine at 21st day of age by drinking water as booster dose) has more protective effects in broiler chicken.

INTRODUCTION

Newcastle disease (ND) is an extremely infectious and generally lethal viral poultry disease infecting mostly chickens, turkeys, and other birds (1). It originated by (NDV); an avian paramyxovirus type1 NDV subdivided into 3 path types according to the virulence in fowls which are: lentogenice, mesogenice and velogenice (2, 3). The clinical signs include respiratory signs such as coughing, panting, sneezing and rales. Additional signs such as falling wings, tedious legs, enlargement of tissues around neck and eyes, twisted neck, rotating and interruption of egg creation (4). Prevention and controlling of the infectious disease in poultry industry by vaccines applied . Consequently, an appropriate strict vaccination program necessity be followed toward prevention the occurrence of clinical sings at farm house level (5). Though, ND immunization programs include both of inactivated and attenuated vaccines to improve protection from infectious diseases. Attenuated vaccines organized with lentogenic strains of NDV and chemically deactivated strains, mixed with adjuvant are commonly used (1,6). In fowl, live vaccines applied by eye dropping or orally to stimulate defensive local immunity managed by (IgA) antibodies (7). While killed vaccine applied by injection has been provide great levels antibodies creating humeral immunity which will defend the chicks from contagion with NDV. Disadvantage of inactivated vaccines are not stimulate mucosal immunity in the respiratory and digestive tracts because oily feature, then the immunity is established slowly. Other disadvantage of it is expensive and hard to applied more than live vaccines (8). Despite of presence of many commercial types of live and inactivated vaccines worldwide, ND is stay a great risk to the fowls trade in developed countries containing Iraq (9). In Iraq, several live vaccines having lentogenic strains of NDV such as LaSota are carried by several importers but efficiency of these vaccines in related with climatic state, dissemination and transport are not every time inspected appropriately and carefully either by the trader or by the handler. Occasionally, the owner are doubtful about the efficacy of those imported NDV vaccines. Many of related questioned are faced the veterinarians in this country such as the immunogenicity, virus titer, stability and like other qualities of those vaccines. Aim of this study is to estimate the efficiency of obtainable ND live and killed vaccine and select an immunization program that will develop thigh immune response.

MATERIALS AND METHODS

Chicks: A whole number of 150 1 day of age unvaccinated mixed-sex birds procured from a local commercial field. Totally birds were cultivated in their cages and nourish and water were provided ad-libitum during the experiment. Beforehand immunization the fowls subdivided in to 5 groups, each one involves 30 birds. The chickens of fifth group were reserved as control without vaccine.

Commercial ND vaccines: Two NDV vaccines are castoff in research. These are Izovac NDV LaSota (live vaccine) and Nobilis ND BROILER (inactivated vaccine) manufactured commercially are bought. By using lentogenic strain La So ta for both immunizing and formulating Ag.

Experimental design: The experimental design as show in Table1. The experimentation are persisted 35 days. The vaccination schedule (created on the local recommended regimens) was as follows:

The G 1: thirty chicks were vaccinated at 7^{th} days old with killed ND vaccine subcutaneously then at 21^{st} days old with attenuated NDV vaccine via drinking water.

The G 2: thirty chicks were inoculated at 7^{th} days old with killed ND vaccine subcutaneously then at 21^{st} days old with attenuated Newcastle disease virus via eye drop.

The G 3: thirty chicks vaccinated at 7^{th} days old with live attenuated Newcastle disease virus vaccine via drinking water then at 21^{st} days old with live attenuated Newcastle disease virus vaccine also via drinking water.

The G 4: thirty chicks were vaccinated at 7^{th} days old with live attenuated Newcastle disease virus via eye drop then at 21^{st} days old with attenuated Newcastle disease virus vaccine via drinking water.

The G 5: thirty chicks were kept as an unvaccinated control.

Table (1). Experimental design with vaccination schedule of different groups

Vaccination day	G 1	G 2	G 3	G 4	G5 (control group)
7th day	ND killed vaccine (S/C)	ND killed vaccine (S/C)	ND live vaccine (D/W)	ND live vaccine (I/O)	unvaccinated
21th day	ND live vaccine (D/W)	ND live vaccine (I/O)	ND live vaccine (D/W)	ND live vaccine (D/W)	unvaccinated

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

Sample collection and sampling schedule: Two ml of blood was obtained aseptically from wing vein or jugular vein of each chick and let it clot and serum parted using bench centrifuge at 1500 rpm for 10 min. The sera were parted and kept at -20 ^{o} C till the serological tests were carried out. Before applied vaccine collected serum as of 25% from chicks castoff randomly on seventh day following hatch to evaluate their maternal immune status. Sera samples are collected before and after 14 days of each immunization and on 7, 21 and 35 days of age from unvaccinated control. Six chick ens were sampled randomly from each group.

Microplate hemagglutination inhibition (H I) test: Microplate H I implemented to determine the antibodies level of the obtained sera as of the bird in the five collections. IgG level of the NDV in the serum was evaluated by H I and cross HI tests in U-bottom micro titer 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 were considered positive as recommended by the World Organization for Animal Health (2).

Chicken interferon gamma (ChIFN-g) ELISA assay: Serum samples were tested to determine the level of ChIFN-g using commercial ELISA kit (Chicken IFN-γ ELISA Kit, Catalog No : E-EL-Ch0026). This ELISA kit applies to the in vitro quantitative determination of Chicken IFN-γ concentrations in serum of chicken. The INF-γ examine was done based on the ELISA manufacturer's protocol.

Lymphoid organ indexes: On day 35 of age, chicks were separately weighted as of each group to evaluate their body mass. Five birds as of each collection were sacrificed. Then a thorough optical assessment, the thymus, bursa of Fabricious and spleen were directly removed, dehydrated then discretely weight up. Later significant lymphoid organ weight variation estimated, their directories were planned (10).

Statistical analysis: A documents collected as of these research in numerous groups were statistically investigated via analysis methods of alteration (ANOVA). $P<0.05$ measured as statistically significant.

RESULTS

Hemagglutination inhibition (HI) test.

The last dilution of antigen which gave hemagglutination was 1:128 therefor the dilution of antigen have 8 HAU was 1:16. This antigen titer (8 HAU) was used in hemagglutination inhibition test. Ab response of the chicks to vaccination patterns were given in Table 2 and figure 1. The mean pre-vaccination H I antibody titer was found to be 4.8 at age 7 days then decline to 2.8 and 0 at age 21 and 35 days correspondingly. HI geometric mean titer (GMT) values after immunization firm and it detected that in 21 day of age (14 days post vaccination) wholly the chicks in the Group 1 to Group 4 sero converted with statistically substantial improved ($p < 0.05$).

H I antibody titers in serum that was protecting. However, the birds in group 4 which had a higher HI titers (179.2). The other groups as the following: 57.6 in group 1, 64 in group 2, 44.8 group 3. Later the supporter vaccination, the GMT standards documented as 230.4 in group1, 256 in group 2, 115.2 in group 3 and 358.4 in group 4 on day 35 of age (14 days post booster vaccination dose). Statistically analyzed of HI GMT and the variance between both was substantial ($P < 0.05$).

Table (2): GMT of Ab titers against NDV in experimental groups measured by HI test

Group	NDV antibody titers		
	At 7 days(maternal immu	At 21 days	At 35 days
Group 1	4.8±1.78	57.6±14.31b	230.40±57.24bc
Group 2		64±0.00b	256±0.00b
Group 3		44.80±17.52bc	115.20± 28.62 c
Group 4		179.20 ±70.10 a	358.40±140.21a
Group 5		2.8±1.09 c	0±0.00c

P≤0.05, small letter describe the significant different between groups.

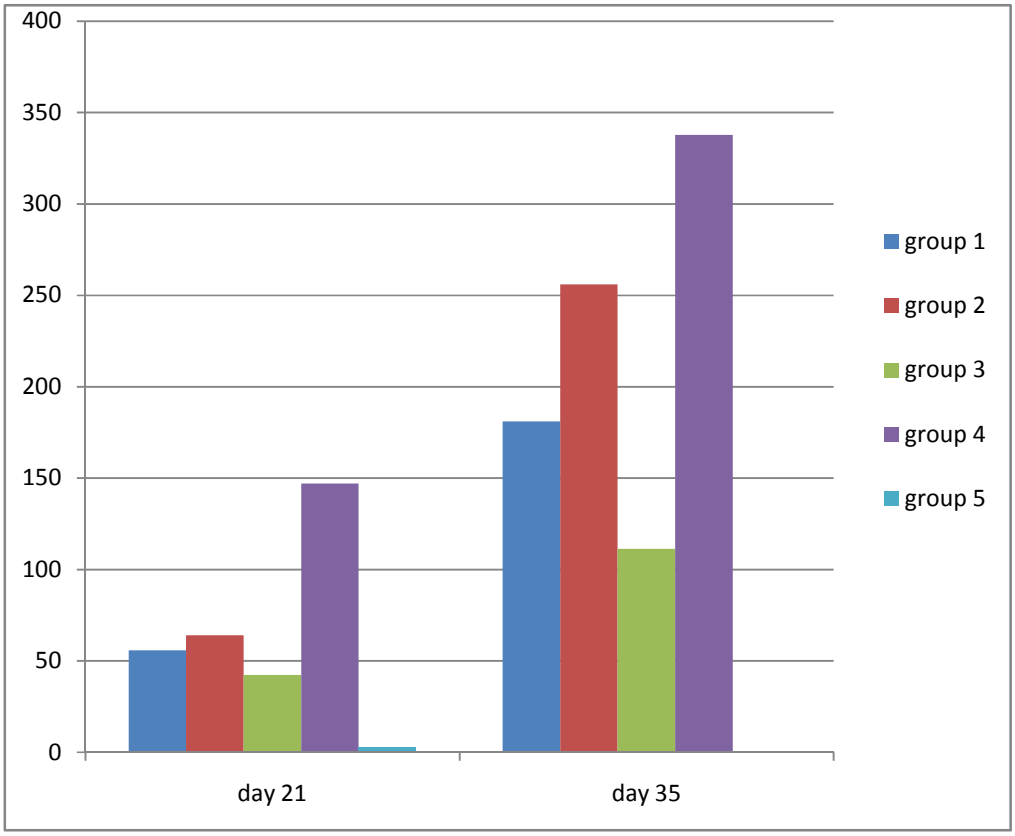

Figure (1): HI antibody titers in experimental groups.

Titer of chicken interferon gamma (ChIFN-γ) production in response to vaccination

The mean ChIFN-γ levels, expressed as pg./mL were presented in Table 3. ChIFN-γ measured at 7, 21 and 35 day-old birds from group 1, 2, 3, 4 and 5 by ELISA revealed that the levels ChIFN-γ were 60 pg./mL pre vaccination (at day 7 of age). On day 14 post vaccination (at day 21 of age), the levels ChIFN-γ were 200 pg./mL in G 1, 180 pg./mL in G 2, 190 pg. /mL in G 3, 250 pg. /mL in group 4, 90 pg./mL in G 5 while on day 14 post booster vaccination dose (at day 35 of age), the levels ChIFN-γ were 250 pg. /mL in G 1, 300 pg./mL in G 2, 230 pg./mL in G 3, 400 pg./mL in G 4, 75 pg./mL in G 5. The levels ChIFN-γ were higher in the vaccinated groups (group 1, 2, 3 and 4) than in the non-vaccinated group (group 5), especially in group 4 (Figure 2).

Table (3): The concentration of chicken interferon gamma (pg./mL) against NDV in experimental groups measured by ELISA test

Group	**concentration of interferon gamma (pg./mL)**		
	At 7 days	**At 21 days**	**At 35 days**
Group 1	60.00 ±14.14	200±28.28a	250±21.21b
Group 2		180±21.21a	300±42.42b
Group 3		190±14.14a	230±28.28b
Group 4		250±42.42a	400±28.28a
Group 5		90±28.28b	75±21.21c

P≤0.05, small letter describe the significant different between groups.

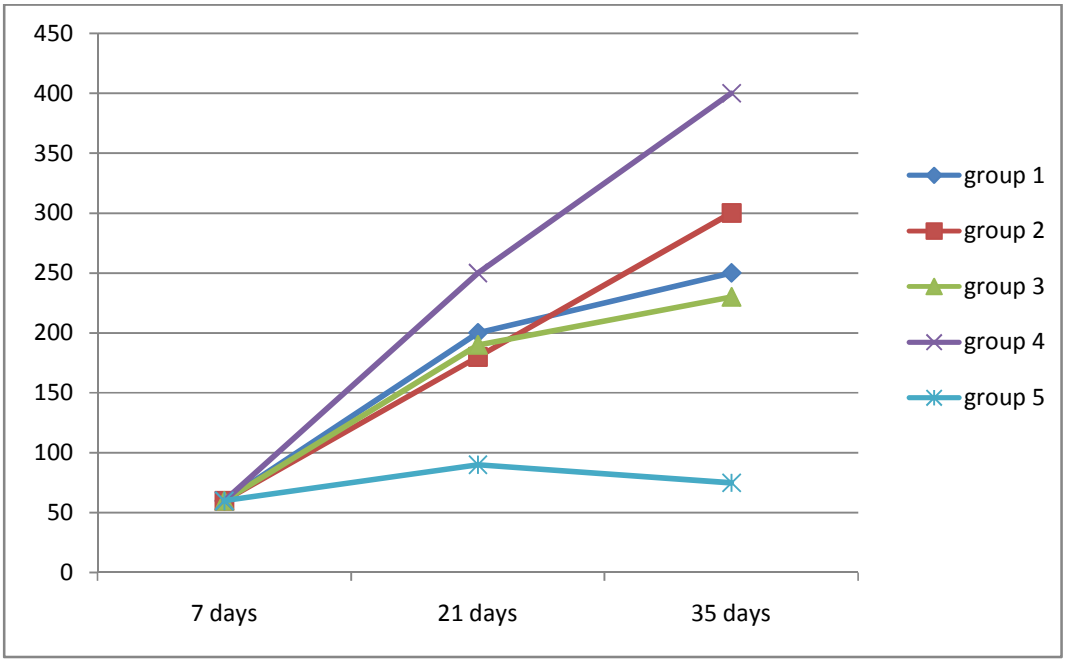

Figure (2): ELISA chicken interferon gamma concentration (pg./mL) in experimental groups.

Lymphoid organs indices: As in Table 4. The highest standards shown the immunized groups in comparing with non- immunized groups at day 35 of age while between vaccinated groups there was no significant different in lymphoid organs indices.

Table (4) Lymphoid organs indices of experimental groups

Group	Indices of Lymphoid Organs at day 35 of age		
	Spleen	Bursa of Fabricious	Thymus
Group 1	0.093±0.005a	0.095±0.02	0.171±0.01
Group 2	0.061±0.01ab	0.086±0.01	0.182±0.01
Group 3	0.077±0.02ab	0.084±0.02	0.197±0.02
Group 4	0.093±0.01a	0.084±0.01	0.194±0.02
Group 5	0.045±0.01b	0.076±0.02	0.142±0.02

P≤0.05, small letter describe the significant different between groups.

DISCUSSION

In the present study, the primary immunization was approved on the time as soon as the parental antibody titer was (GMT 4.8). The results shown that the parental antibody titer in 7th day of old was 4.8, the drop in antibody titer was documented at 21th day of age and reached to undetectable at 35th day of age by estimated using hemagglutination inhibition test diagnostic method for Newcastle Disease.

The incessant risk of ND occurrences in commercial fowls herds requires early protection with conservative ND vaccines managed at an early stages of age. Our study values different vaccination patterns for a commercial presented attenuated vaccines counter to NDV used beneath confined situations. Our results revealed that the Ab titer was diverse considerably between studied groups and best increase antibodies levels was in group 4 which used live vaccine by eye drop at 7th day of age and by drinking water at 21st of age. Our results show that vaccine given by eye drop method is better than drinking water method and live vaccine is better than killed vaccine. This result is also reported in a research that for total vaccines intraocular management improve highest security than drinking water route (11). To hand was diverse vaccines offered for regulatory of N D. Live vaccines are simple applied and relatively cheap and provide good immunity (12). Many investigators have record that attenuated ND vaccines give superior security and healthiness more than inactivated vaccines (13, 14). (15) also other types of N D vaccines managed by eye drop or orally that improved additional mucosal resistance presented by IgA antibodies. The immunity detected in immunized chicks specified that the vaccines were effective. The twofold proliferation in humeral responses of the birds following 'primer dose' and 'booster dose' was detectable with the efficiency of the trade in vaccines. This is in tandem with the work(1,16, 17).

The result of the existing study exposed that antibody tires of diverse immunized groups were significant different. This difference based on the kind of methods of immunization, as a result the cause of high antibody response in group 4 (eye drop rout) of 7 day old provide enough resistance to keep chickens this because attenuated virus vaccine replicates rapidly in mucous membrane of the conjunctiva and nostrils and induce the IgA in the tears (1, 18) such as a cause of local immunity all these details originate from using the attenuated vaccine by eye

drop. The booster dose (orally vaccinated) using for longer-lasting immunity (19). Both humeral and cell-mediated immune responses play important roles in protecting chickens against NDV infection (20, 21).

Therefore in this study we evaluated the cellular immunity as well as antibody titer. Cytokines such as IFN-g are formed by stimulated specific T cells. ChIFN-g capture ELISA more specific and sensitive than the presented bioassay (22). ChIFN-g ELISA as an alternate to the propagation test was inspected after mitogen stimulation of chicks spleenocytes. All vaccinated groups showed high titer of ChIFN-g compared with unvaccinated group. Also group 4 showed higher ChIFN-g titer than other groups. This result also reported by other study that showed maximum of the chicks immunized by the attenuated NDV vaccine formed ChIFN-g later induce stimulus, and this from the time when 2 - 4 weeks after immunization. While other study showed no local correspondence between ChIFN-g produced and humeral reaction (HI titers) could be established after NDV vaccination. The ChIFN-g ELISA has cool prospective for determining the character of cellular immunity for security in contradiction of fowls contagious diseases in the future and will facilitated the research of the role of ChIFN-g in numerous avian immune machineries. The lymphoid organ weight and their indices are beneficial pointers of immunological status (10) and show on the animals' capacity to transmit infection and the providing of lymphoid cells through an immune response (23).The results of our study revealed the immunized fowls have highest spleen directories than non- immunized birds, while other lymphoid organ (Bursa of Fabricious and thymus) showed no significant difference between studied groups. lowest lymphoid organs indices in non-immunized groups referred on little security (24). The clarify in height Ab reaction has been related with a bigger bursa size in White Leghorn chicken strains (25). The available commercial ND vaccine are effective and the best vaccination program for broiler chicken are primary vaccination with live ND vaccine at 7^{th} of age via eye drop followed by booster dose at 21^{st} day of age with live ND vaccine via drinking water. Investigators revealed that infection, shedding, and transmission of virulent NDV in vaccinated birds may occur without clinical signs (26, 27). Agreed this probability we have confidence in that, if defensive immunization pattern are to be employed, they would go composed by a checking program confirming that adequate herd immunity levels are succeeded.

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