

Article

Assessment of Several Newcastle Disease Vaccines' Immunological Effectiveness in Broiler Chicks with Challenge

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Abstract: 125 one-day-old chicks were obtained from the Barakat Al-Zahra nursery in Diwaniyah city and randomly assigned to five groups of identical size. Groups A, B, C, D, and E received vaccinations with several strains included (HB1, Lasota, Clone-30, KomarovR, VG/GA, F) on days 1 & 9 of age, but group E was deemed a control group because it had not received any vaccine. Each group's birds had their blood samples obtained. 13 and 23 day of age. Average antibody titers of NDV Hem agglutination inhibition test (HI) and immunosorbant assay (ELISA) testing following primary and secondary vaccinations. The five groups were exposed to a virulent strain of NDV (Kudu strain 113), 22 day of age by intramuscular injection. The results showed a significant superiority (P 0.05) in the level of antibodies measured in the Hem agglutination inhibition (HI) test and the ELISA test for the group vaccinated with the mesogenic (KomarovR) by injection method and that preceded by a live attenuated vaccine given by spray method. The same group showed very mild disease symptoms that disappeared later, and this group did not record mortality at 0/25 followed by group which vaccinated by live attenuated vaccine by drinking water preceded by dropping. In same time the infection rate increased significantly in the control group following the challenge, this group showed a significant decrease (P 0.05) in antibodies compared to the rest of the groups when this standard was measured at the ages of 13 and 23 days of the birds.

Keywords: ND , vaccines , challenge, infection rate.

Introduction

Newcastle disease virus (NDV), which causes Newcastle disease (ND) in birds, can be filtered. The disease is due to the negative -sense, single -stranded, nonsegmented RNA genome of NDV, an enclosed virus belonging to the Avian paramyxoviridae family and Avulavirus genus [1],[2]. Based on the clinical symptoms observed in infected chickens, APMV-1 strains have been divided into five pathotypes: mesogenic, neurotropic, lentogenic, respiratory infection pneumotropic, and viscerotropic velogenic [3],[4].

Velogenic strains are implicated in severe cases of ND, which is common in many Asian and African nations. Two live vaccine types - lentogenic (B1, F, Clone-30, LaSota & mesogenic (Komarov

strain) as well as Vaccines that are inactivated used in the current ND immunization strategy [5],[6]. Severe forms of the disease cause 90–100% morbidity & mortality making it a global concern with significant economic implications. Both vaccinated and unprotected flocks frequently experience ND outbreaks despite vaccination [7],[8]. This could therefore be the outcome of a failed vaccine. Routine vaccination is used to manage ND worldwide, and the best way to stop disease outbreaks is believed to be proper immunization followed by a strong immune response [9],[10]. Despite the fact that ND vaccination has been used for many years in many nations, The pervasive danger to the poultry industry occurrence of ND outbreaks in vaccinated flocks argued that ND outbreaks in vaccinated flocks could be caused by changes in ND pathogenicity, the strain of ND vaccine virus, and the infectious virus, in addition to the use of insufficient and badly stored vaccines [11],[12]. Additionally, studies have shown that vaccines already on the market offer better protection against viruses identified during previous epizootics in the 1950s than they do against the vast majority of viruses currently circulating in chicken [13],[14]. Additionally, it has been demonstrated that ND-vaccinated hens that are later exposed to virulent strains of the virus can maintain a clinically normal state even if they have the virus in their tissues [15],[16]. Vaccination reduces the quantity of virus released by diseased birds and increases the flock's resistance to infection in 2007. After infecting susceptible hens, research has demonstrated that viraemia plays a critical role in the virus's ability to spread to target organs; however, immunization lowers viraemia and , as a result, virus shedding in chickens [17],[18]. According to the Kudu 113 virus was discovered to be a velogenic pathotype **The aims of this study:** would be to investigate the most effective vaccine in combating this disease and reducing its severity and potential losses because it continues to be a problem in poultry farming and production, and given the importance of this disease in Iraq, as well as the multiplicity of serotypes and vaccines and strains from which the vaccine is made [19],[20].

Material and methods

Experimental Design:

125 Roos breed chicks were brought from Barakat Al-Zahra hatchery in the city of Diwaniyah and were randomly split up into five equal-number groups. A, B, C , D and E. Group A, B, C & D were vaccinated on day 1 and day 9 of the bird's age with lentogenic virulent vaccine strains Volvac B1 Hitchner® (B1 strain), Clone-30, Cevac New L® (LaSota strain), Avinew® (VG/GA) and mesogenic virulent vaccine strains (Volvac Komarov®)The first vaccination was carried out by spraying and the second vaccination was carried out using drinking water .

Table 1. Vaccination Schedule, Vaccine Strains, and Routes of Administration for Experimental Groups.

Age Groups	1 day	Route of administration	9 days	Route of administration
A	Volvac B1 Hitchner® (B1 strain)	Eye drop	Cevac New L® (LaSota strain)	Oral via drinking water
B	Clone-30	Spray	Cevac New L® (LaSota strain)	Oral via drinking water
C	Avinew® (VG/GA strain)	Spray	Volvac Komarov®	Injection
D	F strain	Spray	Cevac New L® (LaSota strain)	Oral via drinking water
E	Un vaccinated	–	Un vaccinated	–

Serological tests :

The antibody standard for Newcastle virus was measured by using two immunological test included HI and ELISA tests on 1 day have been measured the standard of maternal antibodies for the five groups ,13 and 23 days of bird's age .

HI antibody titers are calculated: Each bird had three milliliters of blood extracted from its wing vein, allowed to clot, and then stored in cry tubes. The lyophilized ND vaccination LaSota virus served as the antigen in the HI , which employed a one percent red blood cell (RBC) working solution prepared from ND-free chicken blood. The antigen titer and working dilution were determined using the hem agglutination (HA) test. A modified HI test, as published by (20), was used to measure the antibody titer to NDV. After the frozen sera had thawed to room temperature, the HI test was conducted. 25 μ L of ordinary test serum was serially diluted twice in a micro titer plate. Except for the final well (serum control), each well received 25 μ L of four hem agglutination units (HAU) of NDV antigen. Lastly, fill each well with 25 μ L of (1% chicken) RBC suspension, and let it sit at temperature (35–38 °C) for 45 minutes. The existence of a button at the bottom of each well indicated the presence of positive samples.

ELISA stands for Enzyme-linked Immunosorbent Assay. The amount of NDV antibodies in the serum of vaccinated and unvaccinated chicks was determined using two commonly accessible ELISA kits. ELISA, Part Number: 99-09263, IDEXX Laboratories, Inc., USA, for the detection of NDV antibodies in chicken and turkey serum. 2. The ELISA, product code: CK116 NDV, BioChek BV, Netherlands for the detection of NDV antibodies in chicken and turkey serum. On initial day of the trial, the maternally derived antibody (MDA) was assessed. Each group of hens was given 0.2 ml of an intramuscular solution of the virulent NDV kudu 113 strain with 107 EID₅₀ per ml at 22 days of age. After the challenge, infection and mortality rates were calculated. At the conclusion of the experiment, the number of clinically ill chickens & mortality rates in distinct groups after being challenged with the NDV Kudu 113 strain were computed as a percentage of the beginning bird count. The data was examined using the Statistical Package for Social Sciences (SPSS) software, to find differences between the vaccinated groups, the F-test was used.

Results

On the first day of the trial, the maternally-derived antibody (MDA) measurements revealed consistent mean antibody titers without any statistically significant variations [21],[22].

Table No.1 displays the volumetric standard rate of Newcastle antigen agglutination-inhibiting antibodies in chick serum following the initial and subsequent vaccinations [23],[24]. The results of the statistical study showed notable variations., as the groups were found to be significantly superior on the (C) group (P 0.05) 6.31 ± 0.79 ,. 8.13 ± 0.29 a after the first and second vaccination respectively while the control group E The lowest value by 3.01 ± 0.16 , 1.96 ± 0.18 . In the Newcastle agglutination-inhibiting antibodies, the five groups were significantly superior after the second inoculation over the first inoculation [25],[26].

Table 2. ND agglutinin antibodies that inhibit volumetric agglutination.

Group	standard antibodies Log ¹⁰ (13 days)	standard antibodies Log ¹⁰ (23 days)
A	5.89 ± 0.18^b	7.1 ± 0.34^b
B	5.82 ± 0.22^b	6.45 ± 0.19^c
C	$6.31 \pm 0.79^{a*}$	$8.13 \pm 0.29^{a*}$
D	5.03 ± 0.8^c	6.80 ± 0.99^c
E	3.01 ± 0.16^d	1.96 ± 0.18^d

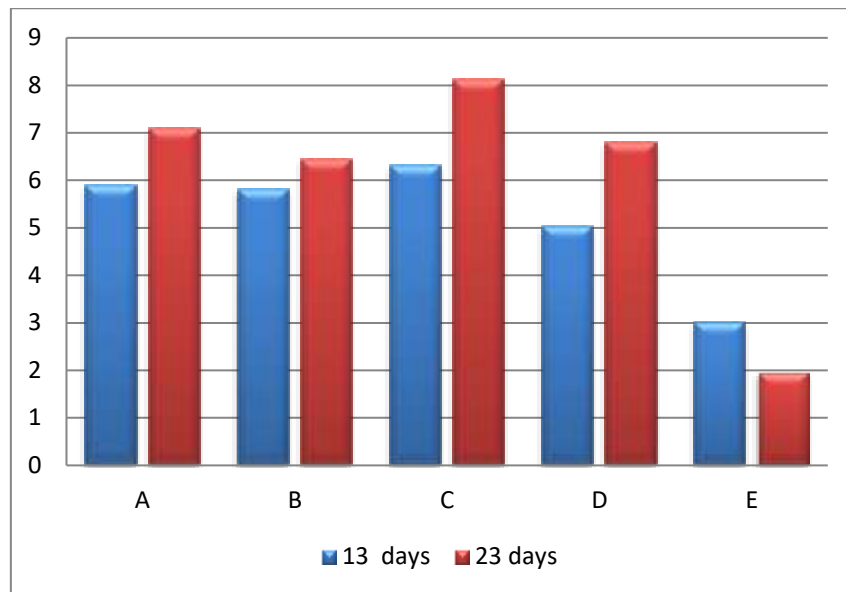


Figure 1. ND agglutinin antibodies that inhibit volumetric agglutination.

Table No. 2 displays the normal volumetric rate of Newcastle antibodies in the experimental ELISA test for groups after the first and second vaccinations [27],[28]. The statistical analysis test results showed that vaccinated groups outperformed the control group after first and second vaccination, with a significant difference ($P < 0.05$). It was also discovered that the five groups performed significantly better after the second immunization than they did after the first. Group C recorded the highest value after the first and second vaccination, by 8.61 ± 0.44 , 9.67 ± 0.22 respectively as for the fifth group, it recorded the highest value among the experimental groups 4.55 ± 0.05 , 3.4 ± 0.19 [29],[30].

Table 3. ELISA volumetric ND antibody titer rate test.

group	standard antibodies Log^{10} (13 days)	standard antibodies Log^{10} (23 days)
A	7.01 ± 0.27^b	8.99 ± 0.19^b
B	6.92 ± 0.22^c	7.85 ± 0.15^c
C	$8.61 \pm 0.44^{a*}$	$9.67 \pm 0.22^{a*}$
D	6.78 ± 0.56^c	7.55 ± 0.45^c
E	4.55 ± 0.05^d	3.4 ± 0.19^d

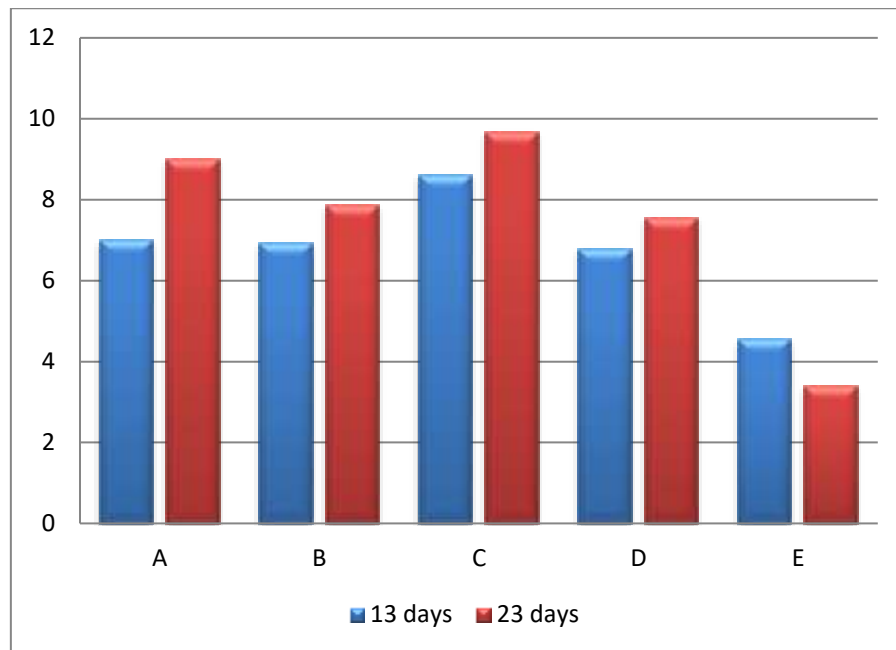


Figure 2. ELISA volumetric ND antibody titer rate test.

Table No. 3 shows the infection rate among the experiment groups, as the results show that there is a clear significant difference between the experiment groups. The fifth group E recorded the highest infection rate, 25/25, while the third group recorded the lowest infection rate, 1/25. The clinical signs appeared to vary in the form and severity of the infection, from mild to severe [31],[32]. The control group E recorded the most severe infection, while the third group C recorded very mild symptoms that gradually disappeared. As for the percentage of deaths, it was found that the highest percentage of deaths was recorded by the fifth group, at 15/25, while the first and third groups recorded the lowest percentage, at 0/25.

Table 4. shows the percentage of infection, clinical signs and Mortality rate among the experiment groups.

Group	Infection Rate	Clinical sings	Mortality rate
A	3/25 ^d	coughing and sneezing, ruffled feathers, listlessness,	1/25 ^d
B	5/25 ^c	coughing and sneezing, ruffled feathers, listlessness .	2/25 ^c
C	1/25 ^e	ruffled feathers, listlessness	0/25 ^e
D	7/25 ^b	coughing and sneezing, rales, ruffled feathers, listlessness ,decrease appetite	4/25 ^b
E	25/25 ^a	20/25 coughing and sneezing, rales, ruffled feathers, listlessness ,loss appetite 5/25 leg paralysis 15/25 diarrhea 3/25 swollen head 4/25 torticollis	15/25 ^a

Discussion

Birds are frequently protected against ND through vaccination. As a result, immunization success is dictated by the vaccine kind, effectiveness, and schedule. Furthermore, the antigenicity of the vaccine virus, the virulence of the field virus, the vaccination procedure, and the age of the birds must

all be taken into account. In Iraq, the most common vaccines used to protect hens from ND are lentogenic and mesogenic live. Aside from these, B1, LaSota, VG/GA, clone-30 and inactivated vaccines are also imported this is confirmed from many researchers such as [33],[34].

Many researchers in this field have found a significant increase in the level of antibodies to the Newcastle virus after several vaccinations, in which the second and third vaccinations are higher than the first vaccination [35].

In this study, we used two important immunological tests to detect antibodies to the Newcastle disease virus, which are the hem agglutination test and the immunological ELISA test, which is considered a sensitive test for these immune antibodies. As for the agglutination test, it is an acceptable test and is within reach, despite the opinions of some researchers in this field that it gives inaccurate results

The increase mean NDV Ab titers 9 days of age was caused by the first active dose of the immunization given day one. There was a clear difference in Ab levels between the vaccinated. The immunogenicity differs when VG/GA , HB1, Clone-30 , F1 vaccinations are used as primers. This is in contrast to, who found no discernible change between the ND vaccination's F-strain, B1, and LaSota strains. Low Ab titers during the challenge period may be the cause of the increase in HI titer for birds in vaccinated groups after the challenge. the group C birds may have benefited from the introduction of the virulent ND virus. This outcome was congruent with the findings who showed an increase in HI titers in hens following challenge with (NDV kudu 113 strain). As a result, it is evident that birds infected with virulent ND viruses may respond by decreasing or raising Ab titers, depending on their immune condition. Following first immunization, the (VG/GA strain) strain had the strongest humoral immune response of any vaccine tested, while the Lasota strain produced substantial antibodies to the Newcastle disease virus. This conclusion is consistent with discovery that the LaSota strain produced more Ab after immunization. The Komarov strain has protected against clinical disease and ND mortality, with a very evident significant difference when given as a second vaccine following the first vaccination with the VG/GA strain, the F, B1, and La Sota of ND were shown to be identical hens vaccinated with Hitchner B1 and Clone-30 .

however, showed a greater HI titre than those vaccinated with F-Strain in terms of Ab production. Consistent with the researchers' findings, the third group vaccinated with the Lasota and Komarov strain vaccine seemed to have a definite moral superiority over the other three vaccinated groups, and the infection and mortality rates in vaccinated chicks seemed to be significantly lower than in unvaccinated chicks. In line with earlier studies by, who found that the VG/GA strain produced stronger immunity than B1, Avinew® (VG/GA strain) had the highest HI titres. It also confirms the results of that birds vaccinated with Avinew® (VG/GA strain) had higher mean HI titres than chickens vaccinated with BCRDV (F-strain). This is in line with.

Each of the three lentogenic vaccines prevented infection with the pathogenic NDV kudu 113 strain and had a satisfactory priming effect. Compared to chickens immunized with the F-strain, those inoculated with Hitchner B1 and Clone-30 primers exhibited a stronger immune response and demonstrated clinical resistance to the disease. Aliyu, 2016. Consistent with earlier studies, the Lasota strain offered noticeably better protection than the B1 vaccines. Three immunoglobulins are produced as a result of humoral immunity being boosted by vaccination against NDV. IgG, IgA, and IgM. Antibodies develop in chicken upper respiratory tract and intestinal tract secretions around the same time that humoral antibodies are found; in the upper respiratory tract, immunoglobulins appear to be mostly IgA with some IgG. IgA is an important protective antibody found mostly in the mucous secretions of the eyes, gut, and respiratory tract, giving local protection to these tissues. Live vaccine strains are the first immunological response to NDV infection and can be detected as soon as two to three days after infection. This appears to account for the early protection against challenge observed in vaccinated birds before a strong antibody response develops. Clinical symptoms, and mortality rates were consistent with previous reports by numerous researchers such as.

Conclusions

Use two doses of the vaccine attenuated by spray ,drinking water and mesogenic virulent vaccines by injection has offered reasonable protection, although to variable degrees, as vaccine (Lasota strain , Avinew® (VG/GA strain, Volvac Komarov®) provided most immunity against the challenge of infection with virulent NDV kudu 113 strain. Additionally, the vaccine using mesogenic strain Komarov® preceded by live vaccine produced considerable effect .We recommend continued research to identify the best effective vaccines for preventing illness spread.

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Conflict of interest

The authors affirm that they have no competing interests.

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