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Assessment of Oil Vaccination Protection to Newcastle Disease Virus Challenges in Layer

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Abstract | The study sought to examine the immunological response of layer hens to a novel strategy of immunization employing an inactivated oil-emulsion of Newcastle disease virus vaccine administered through fine spray. The study also examined the long-lasting nature of passive antibodies in layer hens and evaluated their effectiveness in providing protection against different strains of viruses. There were 200 Lohmann Brown chicks, all 1 day old, which were randomly divided into four groups, each containing 50 chicks. Groups 1, 2, and 3 were administered the vaccination via subcutaneous injection by three distinct commercial businesses at the ages of 8, 70, and 115 days, respectively. Additionally, they were also administered the vaccination through a spray administration on the same day. The control group did not get any vaccinations. Chicken specimens were collected 20 days after immunization, at ages of 30, 50, 70, 90, 110, 130, 150, 170, and after challenging at 200 days. The most pronounced levels of hemagglutination inhibition and ELIZA were observed following the administration of the booster dosages. The p-value over 0.05 indicates that there was no notable difference in antibody production among layer chickens. The study demonstrated that the implemented approach effectively guaranteed the well-being of the herd, leading to optimal productivity without any issues or deaths. Furthermore, the presence of maternal antibodies might be observed till the age of 5 days, suggesting the extent of immunity established before vaccination. Upon conducting a challenge with NDV, the results demonstrated a noteworthy rise in HI and ELIZA titers in the G1 group as compared to G2, G3, and the control group, respectively. In addition, the mortality rate was shown to be 20%, 40%, 50%, and 70% in the G1, G2, G3, and control groups, respectively. In conclusion, the humoral immunity, as indicated by the HI and IgG ELIZA tests, demonstrated that the G1 group, which was vaccinated with the MSD® oil killed vaccine, exhibited the most effective immunization compared to the G2, G3, and control groups, respectively, following exposure to NDV.

Keywords | Vaccination, Newcastle disease, oil vaccine, layers and ELIZA

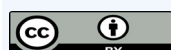
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INTRODUCTION

Newcastle disease virus is a significant concern for most countries worldwide. It monitors and controls high death rates in poultry populations. Several types of

vaccines were produced to combat the illness, including Hitchner B1, La Sota F, K, and inactivated vaccines (Bello *et al.*, 2020). However, Newcastle disease is rather prevalent and can occur even in farms that follow a strict vaccination schedule. The virus poses a continuous threat

to commercial flocks (Eid *et al.*, 2022). The cause of this condition is the Newcastle disease virus (NDV), which is a non-segmented, single-stranded, negative-sense RNA virus. The organism is classified as Orthoavulavirus, which is a member of the Avulavirinae subfamily within the Paramyxoviridae family. This family is categorized within the taxonomic order Mononegavirales (Ahmed and Odisho, 2018). It affects almost all bird species and leads to a variety of clinical symptoms that can range from modest to severe. Chickens have the highest vulnerability among animals, with turkeys ranking second, and geese and ducks coming in third (Okoroafor *et al.*, 2018; Al-Zuhariy, 2023). Vaccination and biosecurity measures are utilized to control and oversee the transmission of the disease. Vaccination, although incapable of inhibiting viral replication and transmission, can offer defense against specific clinical symptoms (Miller *et al.*, 2013). Some individuals may experience a total stop in egg production. Egg producers incur significant financial losses due to the extended duration of these circumstances. Furthermore, a study was undertaken to examine the pathophysiology of exposure to velogenic viscerotropic Newcastle disease virus (vvNDV) in the reproductive system of layer chickens (Al-Zuhariy, 2023). Bwala *et al.* (2012) and Igwe *et al.* (2018) Noticed a substantial decline in egg production in layers that received a vaccination for Newcastle disease (ND). The objective of this study was to assess the immunity of Iraqi Layers chickens (Lohman Brown) against Newcastle disease virus following vaccination with several NDV strains. Eliminated the oil vaccination throughout the manufacturing process.

MATERIALS AND METHODS

The present study conducted at the Al-Hilla Mahaweel Iraq animal facility. The investigation was conducted in a partially enclosed hall that was divided into four sections. Each segment had dimensions of 3 x 2 square meters and was separated by wooden partitions, wire mesh, and individual doors. The sawdust is derived from wood that has a height between 8 and 10 cm. The breeding facilities are fully equipped with essential amenities such as feeders, manholes, and gas incubators. The chicken farming area was equipped with air conditioning, maintaining a consistent temperature of approximately 25 degrees celsius and a humidity level ranging from 60% to 80%. The light cycle followed the pattern commonly observed in a farm setting for commercial activities, with 15 hours of light and 9 hours of darkness. Prior to the commencement of the experiment, there were no discernible indications of disease. One hundred Lohmann Brown chicks from Alkhdraa hatchery were reared in this study. On the eighth day of the experiment, they were split into two equal groups. The first group received an oral ND inoculation at a dose of 0.05ml

on day 200, following genBankMH407204.1 guidelines. Additionally, they were given an inactivated oil killed vaccine three times (on days 8, 70, and 115). The second group (control) also received an oral ND inoculation at a dose of 0.05ml on day 200.

VACCINATION PROGRAM

The birds were immunized against Newcastle Disease (ND) at 8, 70, and 115 days old. The vaccination was administered through an injection of an inactivated oil-killed vaccine. At 12 days old, the birds were given the MA5+ clone30 vaccine using a spray. The ND vaccination was repeated at 70 and 115 days old, with the Clone30 vaccine administered through a spray.

BLOOD COLLECTION

Each group of hens had ten serum samples obtained to conduct an ELISA test and measure the antibody levels. Samples were taken from each group 20 days after the trial commenced. The blood samples were obtained from each bird's wing vein by puncturing it using a 3 mL sterile disposable syringe, following the protocols outlined by Alders and Spradbrow (2001). The blood that was taken was left to clot overnight at room temperature, and then spun in a centrifuge at a speed of 1000 revolutions per minute for 10 minutes. The serum was extracted and stored at a temperature of -20 °C until it was needed for measuring the presence of antibodies utilizing HI assays.

STATISTICAL ANALYSIS

The analysis was conducted using the pre-existing statistical software SAS. The data were presented as the mean value plus or minus the standard deviation. The p-value of less than 0.05 indicated a statistically significant result. The two-way analysis of variance (ANOVA) was employed to conduct numerous comparisons across all groups and across all age categories. The disparities among the coefficients of the experiment were assessed using Dunkin's multi-level test, with a predetermined level of statistical significance set at ($P < 0.05$).

RESULTS AND DISCUSSION

ELIZA TITRATION

To evaluate the humoral immunity (IgG) present in the serum, a total of 150 hens were selected at random and subsequently divided into different groups. The study illustrated how the choice of ND vaccine source contributes to the augmentation of the immune response. According to the data presented in Table 1, there is a consistent and statistically significant difference in IgG titers against ND, with a significance level of $P \leq 0.05$. The group G1 had the highest average antibody titer levels among the immunized groups at 110 and 170 days, with values of 11380.6 ± 462.5

Table 1: ELIZA of ND antibody titer before challenge.

Group age/day	G1	G2	G3	Control
3	4968.1±104.2 Ae	4968.5±108.9 Ae	4968.4±114.2 Ad	4968.1±108.7 Aa
30	7389.6±363.9 Ac	6339.2±331.7 Bd	7002.3±251.2 Ac	1837.7±154.5 Cb
50	5861.8±372.0 Bd	7429.9±386.6 Ac	7364.8±252.3 Ac	622.1±73.8 Ce
70	8823.7±271.5 Ab	7448.2±382.2 Bc	8995.4±384.8 Aa	676.5±39.7 Ce
90	10236.4±571.7 Aa	8476.4±291.5 Bb	9356.3±384.5 Ba	676.5±30.6 Ce
110	11380.6±462.5 Aa	11498.7±491.7 Aa	9763.6±403.8 Ba	661.5±93.7 Ce
130	8181.4±337.3 Ab	7614.4±199.9 Bb	6280.8±218.9 B	954.8±97.1 Cc
150	9604.8±465.3 Ab	7155.6±392.9 Bb	9156.2±486.6 Aa	792.3±37.4 Cd
170	10922.4±461.7 Aa	10079.4±319.9 Ba	8719.2±241.5 Cb	4686.6±238.3 Da

Table 2: Hemagglutination inhibition of ND titer before challenging.

Group Age/day	G1	G2	G3	Control
3	76.8±33.9 A b	100.5±34.2 A b	100.5±34.2 A b	80.7±13.1 B
30	672.3±70.0 A b	585.1±62.9 A b	553.7±66.1 A b	22.6±7.1 B b
50	161.5±56.4 A b	243.5±29.6 A b	240.8±27.9 A b	18.6±4.8 B b
70	201.1±28.4 A b	182.8±15.6 A b	338.2±36.1 A b	10.2±2.5 B b
90	1621.3±68.6 A b	585.1±30.5 A b	1426.2±80.6 A b	6.2±2.1 B b
110	1024.6±71.2 A b	1097.8±88.3 A b	1024.8±51.3 A b	82.2±11.7 B b
130	804.5±73.6 A b	1097.1±90.7 A b	1025.5±52.9 A b	68.5±13.0 B b
150	804.5±29.6 A b	1243.4±180.3 A b	1316.6±71.4 A b	54.8±11.6 B b
170	813.5±23.6 A b	2635.1±132.5 A b	1243.4±80.1 A b	68.5±9.0 B b

Table 3: HI and ELIZA titration post challenged with NDV.

After 200 days	G1	G2	G3	Control
HI after challenge	29482±251.9 A	24166.4±257.2 B	18021.8±242.9 C	16970.2±207.4 D
Elisa IgG after challenge	26620±489.2 A	23266.5±491.9 B	22505.3±499.1 B	18557.4±443.4 C

and 10922.4±461.7, respectively. The group G2 had a moderate average antibody titer level, with high levels of IgG titer at 110 and 170 days, measuring 11498.7±491.7 and 10079.4±319.9, respectively. In addition, the ELIZA titration results of the G3 group showed significantly high levels at 110 and 150 days, measuring 9763.6±403.8 and 9156.2±486.6, respectively. On the other hand, the control group exhibited the lowest amount of antibody titer, in comparison to G1, G2, and G3. G1, which was inoculated with an oily dead ND vaccine, showed higher antibody titers.

HEMAGGLUTINATIONS INHIBITION (HI)

The average HI titer observed throughout the study periods is presented in Table 2. The mean HI values for the G1, G2, G3, and control groups at 3 days old were 76.8±33.9, 100.5±34.2, 100.5±34.2, and 80.7±13.1, respectively. The average HI titer levels of the G1 group steadily grew in accordance with the immunization schedule until reaching a very high value of 1024.6±71.2 after 110 days. The highest value of HI in the G2 group occurred at 170 days, measuring (2635.1±132.5). On the other hand, the G3

group's findings indicated that the highest value of HI was observed at 90 days, measuring 1426.2±80.6.

ELIZA AND HI AFTER CHALLENGING WITH ND VIRUS

At 200 days old the chickens of G1, G2 and G3 were challenged with VNDV strain. The serological results listed in Table 3 and Figure 1, recorded the significant increase at level ($p<0.05$) in the HI titer against NDV in G1 group as compared with G2, G3 and control groups, respectively, the highest mean was given by the G1 group which immunized with oily killed vaccine purchased from MSD company. At the same time, the results of ELIZA revealed that G1 group significantly ($p<0.05$) higher than those titers in G2, G3 and control groups.

CLINICAL SIGNS AND MORTALITY RATES AFTER CHALLENGE

Within the 10-day period following the challenge, the birds who were harmed seemed to be in good health on the day after the challenge. The observed clinical manifestations consisted of decreased food consumption, mild to moderate melancholy, disheveled feathers, and respiratory difficulties

characterized by gasping and sneezing occurring within 10-15 days post conception. Within 10-15 days post-conception (DPC), further indications of anxiety, such as head droop (as seen in Figure 2) and leg paralysis, were also noted. During the ten-day period following the challenge, all hens in the control group exhibited symptoms of illness. The first fatality was observed on the twelfth day after the test, and all unvaccinated chicks perished within thirteen days after the challenge. The data shown in Table 4 indicated that the mortality rate was 20% in G1, 40% in G2, 50% in G3, and 70% in the control group.

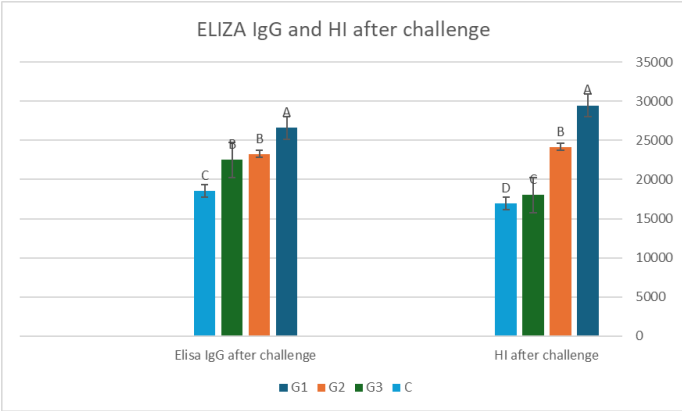


Figure 1: HI and ELIZA titration post challenged with NDV.

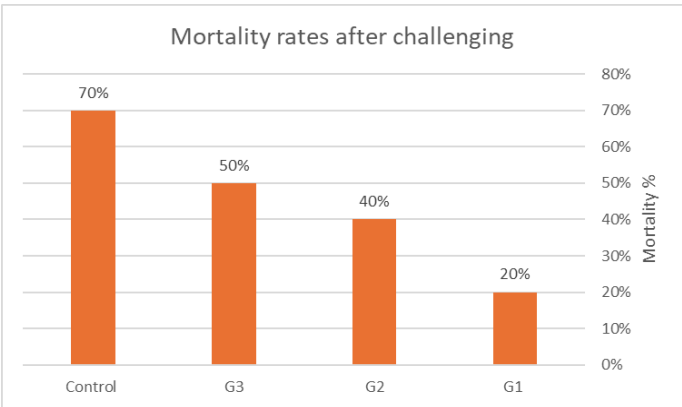


Figure 2: Mortality percent after challenging with NDV.

Table 4: Mortality rate of layer after challenging with ND virus.

G1	G2	G3	Control
10/50 (20%)	20/50 (40%)	25/50 (50%)	35/50 (70%)

The administration of the La-Sota vaccine strain has effectively shielded the birds against death caused by Newcastle disease and reduced the severity of clinical symptoms, pathological alterations, and viral presence in the cloaca (Abd-Ellatieff *et al.*, 2021). This study involved a comparison of oily killed La-sota vaccinations for Newcastle disease, which were obtained from three distinct commercial suppliers in Iraq. To assess the effectiveness of these vaccines in providing protection, we measured the

antibody responses of birds that were vaccinated with different vaccines. We then compared the immune response levels between the vaccinated birds and a control group of birds. This was done by analyzing the average antibody levels for Newcastle disease using the ELISA method and the hemagglutination inhibition (HI) reaction. The results demonstrated that the inactivated oil killed vaccination and clone30 program elicited the most potent immune response when administered via both spray and injection routes. The significance of these discoveries lies in their potential to enhance policies aimed at preventing and managing Newcastle Disease in chicken farms. The avian species had the most robust immunological reaction on the third day, with an average response of 76 ± 33.9411 . Subsequently, there was a substantial reduction in the geometric mean titers seen on days 30 (672 ± 304.0601), 50 (161 ± 56.4168), and 70 (201.1429 ± 68.4). The reduction in GMT values is attributed to the decrease in maternal antibody levels in the chicks as they mature. This suggests that the chicks may not receive sufficient protection against NDV as they mature, even though they acquire passive immunity from the dam (Igwe *et al.*, 2018). Therefore, it is crucial to vaccinate the chicks to enhance their resistance to Newcastle Disease (ND). The findings of Cvetic *et al.* (2021) are consistent with our observations, since they observed the persistence of MDA for 28 days. Furthermore, Ezzulddin *et al.* (2022), Kapczynski *et al.* (2013), and Oberlander *et al.* (2020) have documented that the duration of MDA can extend up to 27 days after birth. Moreover, the results revealed a significant difference ($P<0.05$) in the average HI titer levels between the treatment and control groups. This suggests that the utilization of several oil sources resulted in the death of the ND vaccination, while also enhancing its immunopotentiating impact. This is consistent with previous studies conducted by Hanieh *et al.* (2010) and Landy *et al.* (2011). NDV consists of three nucleocapsid proteins (NP, P, and L), two glycoproteins (hemagglutinin-neuraminidase (HN) and hybrid protein (F)), and protein M (Fukanoki *et al.*, 2001). The protein HN of NDV possesses both hemagglutinating and neuraminidase activities, and it performs a specific role in the disease by enabling the virus to attach to the receptor of the host cell. The hemagglutination inhibition (HI) test is frequently employed in ND serology. Its diagnostic efficacy relies on the immunological response of the poultry vaccination under examination, as well as the prevailing illnesses (Chegrynets *et al.*, 2021). After subjecting the subjects to NDV at 200 days of age, the data shown in Table 3 and Figure 1 indicated that the first group, G1, exhibited greater levels of HI and ELIZA titer compared to both G2 and G3, respectively. As a result, the level of immune response increased over time in all groups. The results indicated that the greater HI and ELIZA mean titer reflected the immunogenicity of the substance and its ability to generate

a protective immune response. Furthermore, the findings of this study indicated that there was a substantial rise ($P < 0.05$) in oil vaccination recipients across all groups, as observed in the results of HI and ELIZA tests. The present results suggest that there is a significant variation in HI titer because of booster vaccination. The findings align with previous data indicating that booster vaccination improves the immune response (Salama *et al.*, 2018). The elevated HI titer following the challenge indicates that the immunizations did not prevent the virus from replicating and being released, but rather decreased these processes. This finding may further corroborate the hypothesis that the utilization of live (particularly LaSota) in combination with inactivated vaccines for the control of vNDV leads to enhanced protection against clinical symptoms, death, and viral shedding. The reason for this is that the immune response against vNDV primarily relies on viral proteins responsible for attachment and fusion, which generate antibodies. Alternatively, it may be mediated by local antibodies, as the humoral titer alone is insufficient for providing enough protection (Kapczynski *et al.*, 2017; Sedeik *et al.*, 2019). Through systematic comparisons, it was observed that all vaccinated groups of chickens achieved a protective level of hemagglutination inhibition (HI) titer against Newcastle disease virus (NDV), as indicated by the GM HI titer $\pm$ SD. Nevertheless, the maternal antibody (HI titer) in the unvaccinated control groups decreased from day 0 to day 35 and eventually reached a level that was not significant compared to the threshold level by the end of the experiment. These findings are consistent with the reports of Banu *et al.* (2009) and Soliman *et al.* (2024) who observed the presence of maternal antibodies up to the 28th day. Several studies have indicated that the efficacy of the Newcastle disease oily dead vaccine is enhanced when the chicks are pre-treated with a live ND vaccine 7-14 days prior to immunization (Lin *et al.*, 1990; Mahboob *et al.*, 1999; Rehman *et al.*, 2002; Waheed *et al.*, 2013). The differences in mean values of HI and ELIZA titration observed in the experimental groups of this study (G1, G2, and G3), which were immunized with commercially available oily killed vaccines from three different companies in Iraq, can be attributed to various factors. These factors may include the quality of the vaccine, the quantity of antigen present, the presence of Baculovirus and/or bivalent antigens, and the manufacturing technology used for the vaccine, which stimulates cellular immunity. These findings are consistent with the results reported by Susta *et al.* (2015) and Sedeik *et al.* (2019). End of text. No clinical indications or deviations owing to the challenge were noted in any of the groups until 10 days after the test. Nevertheless, the control group exhibited clinical manifestations of despair, decreased appetite, anxious symptoms, torticollis, and dizziness within three days after the challenge. In addition, mortality rates of 20%, 40%, and 50% were recorded in

groups G1, G2, and G3, respectively. On the contrary, the results revealed that 70% of the chickens in the control group perished after being exposed to the challenge, whereas the vaccinated groups stayed immune throughout the whole study time. The chickens in groups G1, G2, and G3 had reduced clinical symptoms and mortality rates. The findings indicated that the use of LaSota vaccinations effectively prevented mortality and visible signs of illness in hens after exposure to the disease, which aligns with earlier studies (Liu, 2017).

CONCLUSIONS

Humoral immunity which represented by HI and IgG ELIZA showed that G1 group which vaccinated with MSD® oil killed vaccine gave the best immunization as compared with G2, G3 and control respectively, after challenging with NDV.

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NOVELTY STATEMENT

This study investigated of oil vaccination protection to Newcastle disease virus challenges in layer.

AUTHOR'S CONTRIBUTION

Both authors were involved equally for complete this research.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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