Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2024; 5(1): 238-240
Received: 09-01-2024
Accepted: 15-02-2024

Qayssar Obaid Muhammed
Department of Pathology and Poultry Disease, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Raed Hussain Salih Rabee
Department of Pathology and Poultry Disease, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Corresponding Author:
Raed Hussain Salih Rabee
Department of Pathology and Poultry Disease, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Evaluation of oil vaccines immunity with challenges of Newcastle disease virus in Layer

Qayssar Obaid Muhammed and Raed Hussain Salih Rabee

DOI: https://doi.org/10.33545/27080013.2024.v5.i1c.144

Abstract
The research focused on analyzing the immune reaction of layer hens to an inactivated oil-emulsion Newcastle disease virus vaccine with a new fine spray vaccination strategy, examining passive antibody durability in layer hens, and evaluating protection effectiveness against different virus strains.
Approach: In this study, 200 Lohmann Brown chicks that were 1 day old were divided into two groups of 50 chicks each through random selection. Group 1 was given the vaccine via injection three times at 8, 70, and 115 days old, as well as through a spray on the same day. The control group did not receive any vaccinations. 20 days post vaccination, specimens were collected from chickens vaccinated at 30, 50, 70, 90, 110, 130, 150, 170, and 200 days old.
Findings: All chickens had protective antibodies in 10 serum samples, with the most elevated haemagglutination inhibition titre observed after booster doses. There was no notable difference in antibody production in layer hens, with p value greater than 0.05.
The research demonstrated that the implemented program provided effective protection, leading the herd to achieve peak production with no issues and no deaths. Additionally, antibodies passed down from the mother were present up to the age of 5 days, showing the amount of immunity received before vaccination. During the examination of the deceased birds infected with the virus, distinct lesions like swollen tracheal membranes and petechial and necrotic haemorrhages in the proventriculus were identified.
In conclusion, this research indicates that administering subsequent live virus vaccine through fine spray route immunization is necessary for effectively preventing velogenic viscerotropic Newcastle disease infection. The findings also emphasize the significance of creating successful vaccination strategies and methods to boost defense against ND in layer hens.

Keywords: Haemagglutination inhibition, immunological reactions, protective antibodies, fine spray route, Lohmann Brown chicks

1. Introduction
Poultry farming is crucial for supporting communities globally, especially in low- and middle-income countries, where 70% of all chickens are raised by rural households, contributing to both food and income security (Mujyambere *et al*., 2022) [10]. On the other hand, obstacles come up in the shape of Newcastle disease (ND), a widespread danger that has a substantial effect on chicken numbers, resulting in high death rates in serious outbreaks (Anebo *et al*., 2014) [1]. This illness, a primary factor in global bird deaths, presents a significant danger to chickens, especially in places like Ethiopia, where it is commonly referred to as 'Yedoro Fengil,' due to its sudden onset and severity (Tulu, 2020; Asfaw *et al*., 2021) [14, 2]. Newcastle disease, caused by the Newcastle disease virus (NDV), appears in various forms, with the velogenic strains, specifically the velogenic viscera tropic (VvND) and velogenic neurotropic subtypes, posing major risks to chicken well-being (Samuel *et al*., 2013; Tulu, 2020) [12, 14]. Chickens that are affected by these strains show a variety of symptoms, such as difficulty breathing, decreased egg laying, paralysis, and diarrhea, along with specific postmortem signs of VvNDV. The economic impact is significant, as ND leads to lower productivity and requires a large amount of financial resources for control and vaccinations (Ganar *et al*., 2014; Sedeik *et al*., 2019) [8, 13]. Even with extensive vaccination attempts, recurring ND outbreaks continue to present difficulties for both commercial and rural chicken farms in Ethiopia, as the genetically distinct virulent NDV strains circulating differ from the vaccination strains, creating doubts about the current immunization strategies' effectiveness (Fentie *et al*., 2014; Dimitrov *et al*., 2016) [7, 4].

This research is focused on assessing the effectiveness of the inactivated oil-killed vaccine and clone30 in protecting chickens against NDV, as well as measuring antibody levels through various methods and evaluating protection levels against virus challenge in vaccinated and unvaccinated layer chicken groups while also looking at the persistence of maternally generated antibodies. The results are essential for improving the effectiveness of ND prevention and control tactics in poultry farming settings. The research was conducted in Hilla, Mahaweel, Iraq.

2. Materials and Methods

2.1 Experimental of design

This research was carried out at the AL-HILLA MAHAWEEL IRAQ animal facility. The research took place in a semi-enclosed hall split into four sections, each measuring 3 x 2 m² and separated by wooden partitions, wire mesh, and individual doors. Sawdust made from wood with a height ranging from 8 to 10 cm. It is equipped with all the necessary breeding facilities like feeders, manholes, and gas incubators. The chicken farming area had air conditioning, with a steady temperature of around 25 C and humidity between 60% and 80%. The light cycle mirrored that of a typical farm used for business purposes (15 hours light/9 hours dark). Before the experiment started, there were no observable symptoms of illness. In this study, one hundred Lohmann Brown chicks from Alkhdraa hatchery were raised. 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.

2.2 Vaccination of chicks

The birds were vaccinated against ND at 8,70 and 115 day old was given by injection inactivated oil killed vaccine, at 12 day old MA5+clone30 was given by spray, and repeated ND at 70 and 115 day old Clone30 was given by spray.

2.3 Serum collection

Ten serum samples from each group of chickens were gathered to conduct an ELISA test in order to measure antibody levels. 20 days after the start of the study, samples were obtained from each group on specific days. Blood samples were collected from the wing vein of each bird using a 3 mL sterile disposable syringe, following the methods outlined by Alders and Spradbrow (2001) [16]. The blood that was gathered was left to clot overnight at room temperature, then spun at 1000 rpm for 10 minutes. The serum was separated and stored at −20 °C until it was needed for measuring antibody presence with HI assays.

3. Results

The result of 10 serum samples out of 100 three-day old chicks (before division into groups) for estimation of the maternal immunity against NDV which revealed high level, the mean value was (76±33.9411), which evaluated by HI test.

3.1 Results of antibody response by HI against NDV

The findings of the study showed notable variations at the level (p<0.05) among groups in Ab titer against ND in chicks aged 30, 50, 70, 90, 110, 130, 150, 170, and 200 days old. Nevertheless, the initial group had the highest maternal antibody level of 672±304.0601 among the vaccinated groups at 30 days old chicks, while the control group had 22.6667±21.11 (Table 1).

The findings showed a notable drop in antibody titer against NDV in 50-day-old chicks, with the first group having the highest mean of 161±56.4168, followed by the control group with a mean of 18±22.8736 (table 1).

The findings showed a notable rise in antibody titer against NDV at 70 and 90 days for chicks, with the highest mean in the first group being 201.1429±68.4 and 1621.3333±680.5, followed by the control group with levels of 10.2875±5.5891 and 6.2857±2.1381 (table 1) at the same days.

Nevertheless, there was a notable reduction in antibody titers against NDV in 110-day-old chicks, with the first group showing the highest mean of 1024±512 followed by the control group at 82.2857±44.71 (table 1), as indicated by the results (p<0.05).

While there was no significant difference (p<0.05) in antibody titer against NDV in chicks aged 130, 150, and 170 days, the first group had the highest mean of 804.5714±273.68 at each time point. The control group had lower antibody levels at 68.5714±43.045, 54.8571±35.6, and 68.5714±43.046 respectively (table 1).

On the other hand, the results recorded the significant increase at level (p<0.05) in antibody titre against NDV at 200 days old chicks, the highest mean was given by the first group which was 20482±8851.459, followed by antibody level titre in the control group, which was 16970.2857±12607 (table 1).

Table 1: Results of antibody titer against Newcastle disease (M ± Std) in different days by HI test of the experiment

Age Group /Day	G1	Control
3	76±33.9411	80±39.1918
	A b	B
30	672±304.0601	22.6667±21.11
	A b	B b
50	161±56.4168	18±22.8736
	A b	B b
70	201.1429±68.4	10.2857±5.5891
	A b	B b
90	1621.3333±680.5	6.2857±2.1381
	A b	B b
110	1024±512	82.2857±44.71
	A b	B b
130	804.5714±273.68	68.5714±43.045
	A b	B b
150	804.5714±273.68	54.8571±35.6
	A b	B b
170	804.5714±273.68	68.5714±43.046
	A b	B b
200	20482±8851.459	16970.2857±12607
	A a	B a

* Means ± Std Error

*Means with the same Capital letters in the same Row are not significantly different

*Means with the same Small letters in the same column are not significantly different

3.2 Protection results

During the 10-days period following the challenge, affected birds appeared to be healthy at the day post-challenge (dpc). Clinical symptoms included reduced feed intake, mild to moderate depression, ruffled feathers, and respiratory distress with gasping and sneezing within 10-15 dpc.

Nervous signs, such as wing drop, leg paralysis, were also observed within 10-15 dpc. On the ten day after the challenge, all chickens in the control group showed signs of morbidity, the first death was also noted on the 12 days after the test, and all unvaccinated chicks died within 13 days after the challenge.

3.3 Necropsy results

At necropsy, all of the dead birds had characteristic lesions, such as edematous and diphtheria mucosal membrane in the trachea, petechial and necrotic haemorrhages in the proventriculus, intestine, caecum and caecal tonsils, petechial haemorrhage in the heart and deep-green contents in the gastrointestinal tract starting from the proventriculus, which ended with green faeces.

4. Discussion

This research sought to assess different vaccination schedules for layer hens, employing ND virus vaccines to create a new vaccine program suitable for commercial layer production systems. The findings showed that the inactivated oil killed vaccine and clone30 program had the most effective immunological response through both spray and injection methods. These findings were important for improving strategies to prevent and control Newcastle Disease in poultry farms. The birds showed the highest immunological response on the third day (76±33.9411). Afterwards, there was a significant decrease in geometric mean titers on days 30 (672±304.0601), 50 (161±56.4168), and 70 (201.1429±68.4). The decrease in GMT values is due to the decline of maternal antibody levels in the chicks as they age. This indicates that the chicks may not be adequately protected from NDV as they grow, despite receiving passive immunity from the dam. Hence, it is essential to immunize the chicks to boost their immunity against ND. Our results align with those of Cvetic *et al*. (2021) [3], who noted MDA's persistence for 28 days, as well as Ezzulddin *et al*. (2022) [6], Kapczynski *et al*. (2013) [9], and Oberlander *et al*. (2020) [11], who found MDA lasting until 27 days old. Antibodies in chicks persisted for 31 days after hatching, passed down from their parent stock, reaching highest levels during the layering phase. Transfer of high levels of passive antibodies started at day 5, decreasing gradually but remaining present until day 31 in the control group of layer chickens, as shown by the reducing levels of MDA. Group 1 had the highest GMTs for spray vaccination, with values of 672±304.0601 for the first vaccination, 1621.3333±680.5 for the second vaccination, and 804.5714±273.68 for the third vaccination. Group 1 exhibited the highest Geometric Mean Titers (GMTs) from the vaccines and provided immunity against the virus. The findings indicate that the method of vaccination (spray versus injection) influenced the chickens' immunological response to the ND vaccines.

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