EVALUTION OF THE BEST VACCINAL ROUTES AGAINST NEWCASTLE IN THE PRODUCTION STAGE OF LAYING HENS

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ABSTRACT

Newcastle disease (ND) is a paramyxovirus-based infectious, highly contagious, and pathogenic avian viral disease. Despite the widespread use of ND vaccinations, ND remains a danger to poultry breeders worldwide. The specific goal of this study was to identify the best vaccination route against ND in the layer hens at production stage following oily vaccine. One hundred chickens at 30 weeks of age were collected from layer flocks (ISSA brown) and randomly divided into four groups. The groups received the following vaccinations: G1: Chicks were vaccinated two doses against ND by (*La Sota* strain) through drinking water at (30 and 40) weeks. G2: Chicks were vaccinated two doses against ND by (*La Sota* strain) through cross spray at (30 and 40) weeks. G3: Chicks were vaccinated two doses against ND by (*La Sota* strain) through intraocular at (30 and 40) weeks. G4: Chicks were not vaccinated and consider as control group. All groups challenge with virulent Newcastle virus isolates in a dose ELD_{50} 10^5 at 50 weeks. To measure the (IgG, IgA, and IFN-γ) against ND, blood samples were taken at 35, 45, and 55 weeks of age. According to the results of this experiment, the third group, followed by the second group, produced the highest mean (IgG, IgA, and IFN-γ) titres among the vaccinated groups, while the first group produced the lowest titres when compared to the control negative (fourth) group, which recorded the lowest immune response and highly decrease in eggs production. The results were showed that intraocular vaccination with a live vaccine provides layer hens with a higher level of homogenous protection against vvNDV than spraying or drinking water vaccination.

Key words: ISSA brown, NDV, IgG, IgA, IFN-γ, ELISA.

تقييم افضل طرائق اللقاح ضد مرض النيوكاسل في مرحلة الانتاج للدجاج البياض

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المستخلص

مرض نيوكاسل (ND) هو مرض فيروسي معدي ومعدٍ للغاية ومسبب للمرض (paramyxovirus). على الرغم من الاستخدام الواسع للقاحات ND، إلا أن ND لا يزال يمثل خطرًا على مربي الدواجن في جميع أنحاء العالم. كان الهدف المحدد لهذه الدراسة هو تحديد أفضل طريق للتطعيم ضد ND في الدجاج البياض في مرحلة الإنتاج بعد اللقاح الزيتي. تم جمع مائة دجاجة بعمر 30 أسبوعًا من قطعان البياض (ISSA brown) وقسمت عشوائياً إلى أربع مجموعات. تلقت المجموعات التطعيمات التالية: المجموعة الاولى: تم تحصين الدجاج بجرعتين ضد النيوكاسل بواسطة (لاسوتا) خلال ماء الشرب (30، 40) اسبوع من عمر الطير. المجموعة الثانية: تم تحصين الدجاج بجرعتين ضد النيوكاسل بواسطة (لاسوتا) خلال الرش الخشن في (30، 40) اسبوع. المجموعة الثالثة: تم تحصين الكتاكيت بجرعتين ضد النيوكاسل بواسطة (لاسوتا) عن طريق التقطير بالعين في (30، 40) اسبوع. المجموعة الرابعة: الدجاج لم يتم تحصينه واعتبر مجموعة سيطرة. جميع المجاميع تحدت بالعزلة الضارية لمرض نيوكاسل بجرعة ELD_{50} 10^5 بعمر 50 اسبوع. لقياس (IgG وIgA وIFN-γ) مقابل ND، تم أخذ عينات الدم في 35 و45 و55 أسبوعًا من العمر. وفقًا لنتائج هذه التجربة، أنتجت المجموعة الثالثة، تليها المجموعة الثانية، أعلى متوسط معياري (IgG، IgA، IFN-γ) بين المجموعات المحصنة، بينما أنتجت المجموعة الأولى أقل معيار مقارنة بالمجموعة السيطرة (الرابعة) التي سجلت أقل استجابة مناعية وانخفاض شديد بانتاج البيض. تشير نتائج الدراسة إلى أن التطعيم داخل العين والرش باللقاح الحي يوفر للدجاج البياض أعلى مستوى حماية ضد مرض نيوكاسل مقارنة بالتطعيم بماء الشرب.

الكلمات المفتاحية: ايزا براون، نيوكاسل، IgG، IgA، IFN-γ، الاليزا.

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INTRODUCTION

An acute and widely contagious viral disease known as Newcastle disease (ND) has a devastating financial impact on the poultry business. The disease is caused by Newcastle disease virus (NDV), a non-segmented, single-stranded, negative-sense RNA virus belonging to the genus *Orthoavulavirus* in the subfamily *Avulavirinae* of the family *Paramyxoviridae* in the order *Mononegavirales* (2). It affects almost all bird species and causes a variety of clinical symptoms that might be subtle, mild, moderate, or severe. The most vulnerable animals are chickens, followed by turkeys, and then geese and ducks (9, 19). One of the most commercially significant viral illnesses in chicken is Newcastle disease (15). High mortality rates are a characteristic of the velogenic ND (vND), which can also have an impact on the female reproductive, neurological, digestive, respiratory, and lymphatic systems (5). Vaccination and biosecurity are used to control the disease. Although it cannot prevent the virus from multiplying and shedding, vaccination can protect against some clinical indications (15). The extensive use of live vaccinations makes it challenging to get an accurate evaluation of the distribution of NDV globally. Research has found that ND is widespread throughout numerous nations in Asia, Africa, and the Americas (21). Many vaccinated layer chickens exhibit a reduction in egg production during the peak of the risk period every year, either with or without any clinical indications (10, 18). Some have a total halt in egg production. These circumstances, which might endure for several weeks, cause significant financial losses to egg producers. Furthermore, in a study on the pathophysiology of velogenic viscerotropic NDV (vvNDV) challenge in the reproductive system of layer chickens, Bwala *et al.* (6) and Igwe *et al.* (11) showed a sharp decline in egg production in ND vaccinated layers. The goal of this study was to develop a vaccination route that would provide adequate protection against a decline in egg production caused by vvNDV infection in enzootic areas.

MATERIALs AND METHODS

Experimental animal : At the age of 30 weeks, there were 200-layer chickens in total (ISSA brown). The chicks were raised for two months (March and August 2021) at a nearby farm (Al-Rashidiya Farm) under standard management and biosecurity settings. After cleaning and sanitizing, formalin (37%) and potassium permanganate (in a 2:1 ratio) were combined. At the end of the trial, the pullets were maintained in deep litter with layers of mash and were given unlimited access to water and food.

Vaccination

According to the ISSA accompanying guide, layer chickens were administered the Marek's and (*Hitchner* B1 strain of Newcastle) vaccines at 1 day of age and the *La Sota* vaccine at 3 and 6 weeks of age. IBD vaccinations were given at 2 and 4 weeks, respectively, while pox vaccinations were given at 7 and 12 weeks. At 18 weeks of age, the oily vaccines kill (NDV, IBV and EDS).

Vaccine used in experiment

The live *La Sota* strain vaccination (Intervet-Hollond) was purchased from a neighborhood shop. Following titration of the vaccine, each Dosage was determined to contain $10^{6.5}$ embryo infective dose (EID_{50})/ml by Hemagglutination test (HA)

Challenge study

All chickens in each group were challenged with virulent NDV (ICPI=1.86) individually at the age of 50 weeks. Each chicken was given an oral drop of around ELD50 10^5 of pathogenic NDV (25). Five weeks after vaccination, clinical symptoms (egg production) and death were noted post infection (PI).

Sample collections

Five birds from each group's jugular veins were drawn at 7, 14, 21, 28, and 35 days to separate the serum, which was then used in an ELISA assay to evaluate (IgG, IgA, and IFN-γ) titre.

Serological analysis

Jugular vein blood was extracted, allowed to clot, divided, and frozen at -20 °C until needed. The ProFlock® ELISA kit (*Synbiotics*-USA) manufacturer's instructions were followed for performing the procedures for the indirect (ELISA) test used to detect IgG, IgA, and IFN-γ in chickens.

Statistical analysis

Using the SAS statistical analysis system, a one-way ANOVA was performed on the data

(23). The difference between the means (P<0.05) was found using the least significant differences (LSD) method.

RESULTS AND DISCUSSION

Humoral immunity detection (IgG)

In order to assess the humoral immunity (IgG) in serum, 10 hens were randomly chosen and then separated into groups at 30 weeks. The results were 5360.9±288.7. The study demonstrated how vaccination regimens help to enhance the immunological response to ND. As shown in (Tab.1), IgG titres against ND are significantly different at the level (P≤0.05) at all times. The highest mean antibody level of titre among the immunized groups was given by G3, followed by a medium mean antibody level titre in the G2 group. In contrast, the lowest level antibody titre was given by G1 when compared with the control negative G4 group, which recorded a significant decrease in IgG against ND, which is similar to the last oily killed immunity, G4 increased significantly (P≤0.05) after vvNDV challenge at 50 compared to vaccinated (G3, G2, and G1).

Table 1. Statistical data describing the IgG ELISA titre against ND

Groups	35 weeks	45 weeks	55 weeks
G1	6154.5±456.2 C	7586.8±987.4 C	5786.2±698.2 D
G2	7121.4±321.3 B	9657.4±786.1 B	10876.9±897 C
G3	8453.7±212.3 A	11437±546.6 A	12564±765 B
G4	4216.3±645.3 D	2312±788.1 D	18379.7±5460 A
LSD	512.28	755.9	876.6

Five samples were collected. Capital letters denote a significant difference at the level of (P≤0.05).

According to this study, triple *La Sota* vaccinations can effectively protect chicken layers against all clinical symptoms, including a decrease in egg production, for at least three months. It should be noted that this runs counter to the majority of producers' present routine, which involves one *La Sota* revaccination every three to six months. But it should be noted that in other areas where different vvNDV strains are enzootic, this vaccination campaign may not be sufficient. Data also indicated a progressive increase in antibody titer as the weeks passed. According to the comparison, the drinking water route was the least effective of the three immunization strategies. It could be that the intraocular and intranasal routes are more effective methods of vaccination, but the antigens supplied by this route are occasionally lost before ingestion or partially or completely digested in the alimentary canal. According to the findings, eye drop vaccination (G3) produced a higher level of ELISA titer and protection than oral drop vaccination (G1), which is consistent with an earlier publication in which the authors evaluated similar means of administering the NDV vaccine (1). The outcomes had a clear connection to the vaccine route. During an eye drop vaccination, the vaccine virus has an opportunity to activate lymphoid cells in the Harderian glands, which are situated at the median side of the eyes, to create local antibody responses such as IgA and lacrimal IgM (22). The vaccine virus, however, may have a chance to enter the digestive tract after immunization by oral drop and may be eliminated by stomach secretion (27). According to Kafi *et al.* (13), secondary NDV immunization produced the highest titres, which were noticeably higher than those from a single vaccination. The age was chosen because layers are more vulnerable to infections at this age due to the stress that comes with egg production. In this study, the layers received four doses of the *La Sota* vaccination (4). In comparison to groups that had received vaccinations, G4 had the greatest levels of IgG after being exposed to the vvNDV. These findings are consistent with those of Igwe *et al.* (11), who linked high levels of IgG to mortality following vvNDV exposure in layer hens.

Local immunity detection (IgA)

Using an ELISA, serum local immunity (IgA) at 30 weeks was measured to be 76.2±13.7. Table (2.) shows statistically significant differences at the level (P≤0.05). The G3 group had the greatest IgA titre among the vaccinated groups, followed by the (G2 and G1) groups at weeks (35 and 45), where there was a significant rise in IgA at all levels

(P≤0.05). Compared to the control negative G4, which saw a large drop in IgA against ND, similar to the last oily destroyed immunity, G1 had the lowest antibody titre. G4 increased significantly (P≤0.05) after vvNDV challenge at 50 compared to vaccinated (G3, G2, and G1).

Table 2. Statistical data describing the IgA ELISA titre against ND

Groups	35 weeks	45 weeks	55 weeks
G1	92±21 C	172.5±28.1 C	186.4±63.1 C
G2	122.3±43.3 B	233.1±41.5 B	275.8±34.6 B
G3	178.8±56.3 A	329.4±33 A	366.4±56 A
G4	62.4±11.3 D	46.7±14 D	432.8±9.3 A
LSD	24.2	52.6	94.3

Five samples were collected. Capital letters denote a significant difference at the level of (P≤0.05).

The respiratory and digestive tracts of birds are part of their mucosal immune system. While parenterally delivered vaccinations mainly trigger systemic reactions, mucosal immunization promotes mucosal immune responses with S-IgA antibody as well as systemic humoral and cellular immunological responses (17, 29). When compared to other vaccination methods, mucosal delivery offers a number of benefits, including a wide epithelial surface with a lot of microvilli, a porous endothelium membrane, a highly vascularized mucosa that facilitates absorption, and ready accessibility (28). Following the first vaccine, the amount of IgA increased by six times, and this pattern persisted after the second vaccination by two times. Contrary to broiler chickens, layer hens' antibody levels did not drop following vaccines (30). It has been shown that NDV-specific antibodies can be found in chicken blood, peaking 21 to 28 days after the live virus vaccination or the first week following the infection (14). The IgA titers increase significantly, reached their peak at day 28 post vaccination, and didn't significantly decline until day 42. Additionally, the oral mode of administration cannot be used with the *La Sota* strain of NDV vaccination due to its strong affinity for respiratory system cells (12). While just a small amount of the vaccine is inhaled during spray vaccination and infects susceptible cells, a sizable portion of the vaccine ends up on feathers or the ground (8). The highest IgA was seen in G4 following the vvNDV challenge compared to the vaccinated groups; these findings are consistent with those of Waheed *et al.* (30), who observed a high IgA level with mortality following the vvNDV challenge of layer hens.

Cellular immunity detection (IFN-γ)

Serum cellular immunity (IFN-γ) was measured using ELISA at 30 weeks was 22.8±6.2. IFN-γ levels significantly changed across all periods (P≤0.05). (Tab. 3). Compared to the vaccinated groups, G3 had the greatest IFN-γ levels, G2 had a medium level, and G1 had the lowest levels. When compared to the control negative group G4, which had a large decline in IFN-γ against ND, reflecting the final phase oily killed vaccine. After the vvNDV challenge at age 50, G4 significantly (P≤0.05) increased in comparison to the vaccinated (G3, G2, and G1).

Table 3. Statistical data describing the IFN-γ ELISA titre against ND

Groups	35 weeks	45 weeks	55 weeks
G1	27.3±122.2 C	34.1±14.2 BC	40.4±24 C
G2	36.8±14 B	47.4±16.1 B	63.2±18.6 AB
G3	44.6±16.2 A	61.7±13.5 A	70.7±22.6 A
G4	18.2±8.7 D	13.5±9 C	89.3±6.7 D
LSD	6.4	12.7	14.5

Five samples were collected. Capital letters denote a significant difference at the level of (P≤0.05). Inflammatory cytokines are essential as natural immune response regulators and mediators (3). When chickens are infected with intracellular pathogens, Th1-type cytokines (IL-2, IL-12, and IFN-γ) prevail and generally boost cellular immunity (20). Because cytokines are required for the development of cellular immunity and the

prevention of viral infection (16), IFN-γ production levels, a sign of a Th1 response, are crucial cytokine levels. Higher IFN-γ levels were found in the G3 of chickens who received intraocular and intranasal vaccinations in G2. The cellular immune response is best understood through IFN-γ expression (31). The findings of the present study are consistent with those of Dalgaard *et al.* (7) and Sharma and Rautenschlein (26), who reported higher IFN-γ expression after intraocular revaccinations of the *La Sota* strain. The highest IFN-γ was detected after intraocular vaccination, followed by intranasal vaccination in G2.

At 5 weeks after infection, number of dead birds 44% in the control group (G4). Groups 3, 2, and 1 that had received vaccinations had protection rates of 100, 88%, and 76%, respectively. These protection rates were significantly greater than those of the unvaccinated (G4) group. The protection rate of hens who were administered the vaccination intraocular (G3) was higher than that of chickens who received the vaccine intranasally and orally (G2, G1) (Table 3). The vaccine group, which recorded a slight decrease in egg production, especially in G1, in comparison with G4, which revealed a more significant decrease in egg production.

Eggs production and protection rate

Table 4. Mortality, protection and Eggs production at 55 weeks post challenge with vvNDV.

Groups	Mortality		Protection		Eggs production	
	Number	Percent	Number	Percent	Number	Percent
G1	6/25[b]	24	19/25[c]	76	15/25[c]	60
G2	3/25[bc]	12	22/25[bc]	88	18/25[b]	72
G3	0/25[c]	0	25/25[a]	100	22/25[a]	88
G4	11/25[a]	44	14/25[d]	56	10/25[d]	40

Five samples were collected. Small letters denote a significant difference at the level of ($P \leq 0.05$).

In contrast to a previous report of just 67%, the protective effectiveness of hens in G3 who got the vaccination by eye drop in this trial was 100%. (24). The age was chosen because the stress of producing eggs makes the layers more susceptible to infection. The layers in this study received four doses of the *La Sota* vaccination (4). The findings concur with those of Igwe *et al.* (11) who observed a sharp decline in egg production following the vvNDV challenge of vaccinated layers. The results of each study's comparison may change, most likely as a result of variations in the study's specifics, such as the quantity or virulence of the challenge virus. When egg production was at its highest, triple revaccinations led to high antibody titres that provided protection for longer periods of time than single revaccinations, according to Bwala *et al.* (6).

CONCLUSIONS

According to the study's findings, live *La Sota* strain NDV vaccination given to hens by eye drop administration resulted in a greater antibody response and improved defense against the NDV challenge than did the same vaccine given to chickens via intranasal and oral drop administration.

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