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Various Immunization Routes, Times and Types to Prevent Newcastle Disease

Ali S. A. ALHassan, Nafea S. Jasim
University of Al-Qadisiyah, veterinary collage, Pathology and Poultry Diseases department, Iraq

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Abstract: We adopted different methods and types for administering the vaccine against Newcastle disease to 175 Ross 308 breed newly hatched chicks, one day age. First group G1 was vaccinated using eye instillation on the 1st day and then the same vaccine was repeated in the same way on 8th day and the vaccine was given to the second group(G2) in the same way but it was returned on 15th day of age .The third group (G3) was given the vaccine by spraying on 1st t day and revaccinated oil emulsion sub cutaneous on 5th days It was boosted with a second live vaccine by drinking method at 15th day age.

The fourth group (G4) was vaccinated with drinking water on 10th day of the bird's age and it was returned after ten days, leaving one group of them without vaccination, it was used as a control group (G5). Six group (G6) vaccinated with double vaccine (H9N2-ND) vaccine while group seven (G7) immunized with ND rec .on MD at first day . ELISA test was performed to measure the size of the antibodies at different times of the bird's age. The volumetric standard for antibodies resistant to the Newcastle disease virus of G3 increase significantly ($p<0.0001$) at value 5867 ±261.4 , 8980. ± 498.6 in 14th days and 24th day respectively .

G1 that was given the vaccine by dropping in the eye on 1st day and repeated on 8th days increase significantly ($p<0.0001$) a value of 6898.2±347.1

compared with dropping in the eye 1st day and returned on 15th days (G2) of age 6758.7 ±232.8 at value .G4 that giving live vaccines using the drinking water method was gives results, but with a lower percentage of protection than the two mentioned methods with a value 5880.4±476.7 when measuring the volumetric antibodies standard on 24th day respectively of the bird's age.

Live vaccines in all ways increase significantly ($P < 0.0001$) volumetric antibodies titer means measured on 24th day than measured on 14th day and increase significantly ($P < 0.0001$) than control group (G5) which had a significant decrease ($P<0.0001$) means of the (705.2 ±505.01) on14th day to (313.1 ± 560.7) on 24th days.

On other hand the recombinant vaccines for Newcastle disease (ND rec .on MD) vaccinated group increase significantly ($P<0.0001$) over two vaccinations dropping methods at different times, injection killed vaccine alone and two vaccinated drinking water method at different times with a value 7211.6 ± 550.2 .

Double vaccine H9N2 –ND gave good results and did not affect the health of the birds, as a value of (6572±572.5) was recorded when measured on the 24th day of the birds' age.

Key words: Newcastle disease, Live vaccine, Inactivated vaccines, Recombinant vaccine Double vaccines, Different routes and times, ELISA test.

Introduction

All bird species are susceptible to Newcastle disease (ND), a deadly and highly contagious virus. It is found all over the world and poses a serious threat to the poultry industry since it causes enormous financial losses, especially in chickens and turkeys (Haque et al., 2010) (1). Avian Paramyxovirus type-1 (APMV-1) is the causative agent of Newcastle disease (ND) and is a member of the Avulavirus genus, Paramyxovirinae subfamily, Paramyxoviridae family, and Mononegavirales order (Ghiamirad et al., 2010) (2). According to the clinical signs and symptoms observed in infected chickens, five pathotypes of APMV-1 strains have been identified (Alexander & Senne, 2008) (3). They are subclinical, mesogenic, lentogenic or respiratory infection, neurotropic velogenic, and viscerotropic velogenic. Protection from virus infection and replication would come from vaccination against NDV (Alexender, 1997).(4). Despite the fact that ND immunization typically shields the bird from the more severe effects of illness, virus shedding and multiplication

might still happen, creating an infection source (Chukwudi et al., 2012). (5). Live Vaccines: There are two categories of ND virus strains utilized in commercial live viral vaccines: Lentogenic vaccinations, include strains of Lasota, V4, Clone 30, F, and Hitchner B1. Roakin, Mukteswar, and Komarov strains are examples of mesogenic vaccines (OIE, 2004). (6) B1, F, and Lasota are among the lentogenic strains that are utilized on very early chicks without harming the host. They can be given by injection in eggs, beak dipping, spraying, eye drops, or intranasal drops. (Cho and others, 2008) (7).Inactivated Vaccines: To make the inactivated virus more immunogenic, a carrier adjuvant is often added to infectious allantoic fluid that has been treated with formalin or propiolactone to kill the virus (Grimes, 2002) (8).The significance of ND vaccine and vaccination program evolution is underscored by DNA recombinant vaccine: ND outbreaks (Kapcynski and King, 2005) (9.). A quick serological method for identifying NDV antibodies in chicken serum samples is the ELISA test (Chaka et al., 2013) (10).

Material and methods

Experimental chicks

175 Ross 308 breed newly hatched chicks, one day age.

Experimental chicks source

The chicks were brought from the Middle East hatchery/Mazraa district/AL-Saouira/Wasit.

Experimental place

The chicks were placed in special room designated for this experiment, where reservations were made to isolate each group from the other group.

Experimental chicks Groups

The chicks were distributed randomly into (7 groups) with 25 birds per group .

Experimental chicks vaccination

- Groups were vaccinated with vaccines against Newcastle disease in different rout ,times types as shown in this table .

Groups of chickens vaccinated against Newcastle disease.

Vaccine day	G1	G2	G3	G4	G5	G6	G7
1 day	Lasota ND vaccine Dropping	Lasota ND vaccine Dropping	Lasota ND vaccine Spray		Distilled water injection	H9N2 - ND Killed vaccine	ND. Recombinant on Marek D.
5 days			ND vaccine Injection				
8 days	Lasota ND Dropping				Distilled water injection		
10 days				Lasota ND Drinking water	Distilled water injection		
15 days		Lasota ND Dropping	Lasota ND Drinking water		Distilled water injection		

20 days				Lasota ND Drinking water	Distilled water injection		

(G1, G2, G3, G4, G5) were examined at 14 and 24 days of age by ELISA test.

G6 and G7 were examined at 24 days of age by ELISA test was performed in the (Dr. Mustafa Al-Jaff veterinary lab. Iraq – Baghdad- Al-Sinak Specialized for diagnosing poultry diseases)

Newcastle disease vaccines

ND killed - (H9N2)

Name: vaxxon ND-flu /500mlm , dose: 1000 does 2500 dose (broilers)

ND Recombinant on disease:

Marek's disease -Newcastle disease vaxxine Serotype 3.live marek's disease vector

Dose 0.2Poulvac ® proceeta (tmc) HVT-ND /frozen by N2/ 2000 doses.

ND In activated vaccine

Emulsion for injection for broilers laying hens and breeders Keep in orginal package in order in protect from light once opened use with. In 10 hr . 500 ml , Dose 2500 (Broilers) Vaxxon ND_Flu®, Compenent Vaxxinova

ND lasota :Vaxxon lasota (live vaccine)

Batch 1369 p ,Vaxx inova international B.V Netherlands, doses 2500 (broilers).

Vaccination methods

Drinking water method

The live attenuated Newcastle vaccine containing 1000 doses of 5 ml of clean water was mixed, and a volume of (0.5) ml suspension was withdrawn and mixed with a volume of 99.5 ml of clean water. A sufficient quantity was taken from the mixture according to the number of chicks and their ages:-

The amount of water used for vaccination (ml) = the age of the chicks in days x the number of chicks in the hall.

Vaccination was done according to the vaccine schedule above after thirst. the chicks were thirsty for two hours, after which they were given water containing the vaccine.

Spray method

The live Newcastle vaccine (containing 1000 doses) was mixed in 5 ml of clean water, then mixed well. A volume of 0.5 ml of suspension was withdrawn and mixed with a volume of 99.5 ml of clean water. A sufficient quantity was taken from the mixture. The chicks were vaccinated with rough spraying after turning off the lights and closing the doors and windows. and air dischargers and the chicks were vaccinated using a special sprayer for this purpose. Droplet size ranges from 50 micron and is at a height of 50 cm from the surface of the cage containing the birds.

Injection method

Use the oil vaccine at a rate of 0.5 ml. for each bird, according to the manufacturer’s instructions, by injecting it under the skin and behind the neck using an injection gun **.**

Dropping method

The live Newcastle vaccine, containing 1000 doses, was mixed in 5 ml of clean water, then mixed well. A volume of 0.5 ml was withdrawn from the suspension and mixed with a volume of 99.5 ml

of clean water. A sufficient quantity was taken from the mixture and vaccinated by instillation in the eye of the bird 0.02 mL at dose **.**

Blood collection

Ten samples of blood were taken from each group, and the blood was extracted straight from the heart, put in test tubes, and left at an angle for an hour. The glass tubes containing the blood were then placed in the refrigerator vertically for 18 to 20 hours, and finally the serums were extracted after the centrifugation process. To perform the ELISA test, the serum must first be separated at a centrifugation speed of 3000 rpm. It must then be stored in plastic containers labeled with the groups' names. Until it was used, the serum was stored in the freezer.

Antibodies titer

Following that, blood is extracted to check for antibodies in the serum, which is one of the most crucial aspects of Newcastle disease viral agglutination with red blood cells and is used to assess the humoral immune response of birds against Newcastle disease in the bird serum samples.

Procedures of indirect ELISA for NDV

As stated by OIE (2018) (11) , the test was conducted in accordance with the manufacturer's instructions found in (Dutch biojack® NDV Holand ELISA kit).

Results

Volumetric Newcastle antibodies titer percentage ELISA test measured post vaccine **.**

Table No.1 Show the percentages of the Newcastle antibody titer measured by the ELISA test, the results show that seven groups vaccinated with Newcastle vaccine and by all methods were 100% positive, whether on day 14^{th} or day 24^{th} of the bird's age while the control group (G5) recorded 35% , 19% on 14^{th} , 24^{th} day respectively .G6,G7 were 100% positive at 24^{th} day .

Table (1) Volumetric Newcastle antibodies titer percentage ELISA test measured post ND vaccine.

Groups	vaccine rout	Vac. age	ELIZA test day	Positive No. Antibodies titer > 732
G1	Dropping1	1^{st} day and re-vaccinated 8^{th} day	14^{th}	%100
			24^{th}	%100
G2	Dropping 2	1^{st} day and re-vaccinated 15^{th} day	14^{th}	%100
			24^{th}	%100
G3	Spray and re vac. sub cut. + DW	1^{st} day spray re-vaccinated oil emulsion 5^{th} day sub cut. 15^{th} day D.W	14^{th}	%100
			24^{th}	%100
G4	Drinking water	10^{th} day 20^{th} day	14^{th}	%100
			24^{th}	%100
G5	Control	-	14^{th}	%35
		-	24^{th}	%19
G6	H9N2-ND		24^{th}	100%
G7	ND rec .on MD		24^{th}	100%

Negative immune status (ELISA Antibody titer) < 732 , S/P value Immune < 0.5

Positive immune status (ELISA Antibody titer) > 732 , S/P value Immune > 0.5.

Volumetric Newcastle antibodies titer mean ELISA test measured post Lasota vaccine.

Table No.2 and Figure No. 1 show the rate of Newcastle antibodies in the serum of chicks after vaccination with the Newcastle vaccine Lasota strain in different ways and at different times measured by the ELISA test at 14 and 24 days of age.

The results show a significant increase P<0.0001 in the means of the volumetric antibodies titer for the G3 which vaccinated by spray and sub cutaneous method and boosted with another live vaccine (5867 ±261.4) at value and (**8980. ± 498.6**) on 14th , 24th day of age respectively .

G1 which vaccinated by instillation in the eye on 1ST day and re-vaccinated on 8th day of the bird's age increase significantly (P<0.0001) (6898.2 ±347.1) at value than G2 vaccinated using the dropping method on the first day and re-vaccinated on 15th day of the bird's age (6758.7 ±232.8) at value which measured on 24th day of age and recorded (5116.3 ±565.8) at value and recorded (4120 ± 177.8) on 14th day of age

G4 vaccinated with drinking water showed a good result, when the antibodies were measured on the 24th day of the bird's age , with a value of (5880.4 ±476.7) but decrease significantly (P<0.0001) in the means of the volumetric antibodies titer of G1,G2,G3 .

The results show all vaccinated groups G1,G2,G3,G4 significant increase P<0.0001 in the means of the volumetric antibodies titer on 24th day than the means of the volumetric antibodies titer on 14th day that vaccinated with different ways.

(G5) which is the control group had a significant decrease (P<0.0001) means of the volumetric antibodies titer on 14th day with a value 705.2 ±505.01 to 313.1 ± 560.7 on 24th day.

Table (2) Volumetric Newcastle antibodies titer mean ELISA test measured post vaccination.

Group	vaccine routs	Vaccination age	Ab titer (mean ±SE) 10^{log10} (titer) 14 days	24 days
G1	Drop 1	1st day and repeated on 8th day of age	5116.3^{Bb} ±565.8	6898.2 ^{Ba} ± 347.1
G2	Drop 2	1st day and repeated on 15th day of age	4120 ^{Db} ± 177.8	6758.7 ^{Ca} ±232.8
G3	Spray and sub cut. Injection+ DW.	1st d. sp. and vac oil sub cut. on 5th d. of age boosted with live DW. on 15th d. of age	5867 ^{Ab}±261.4	8980. ^{Aa} ± 498.6
G4	Drinking water	10th day and repeated on 20th day of age	4388.1 ^{Cb}± 711.3	5880.4 ^{Da}±476.7
G5	Control	–	705.2^{Ea} ±505.01	313.1 ^{Eb}± 560.7
P value			P<0.0001	
LSD P<0.05			1101.7	

* Capital letters represent the vertical statistical reading (between totals), while lowercase letters represent the horizontal statistical reading (between times).

Negative immune status (ELISA Antibody titer) < 732 , S/P value Immune < 0.5

Positive immune status (ELISA Antibody titer) > 732 , S/P value Immune > 0.5

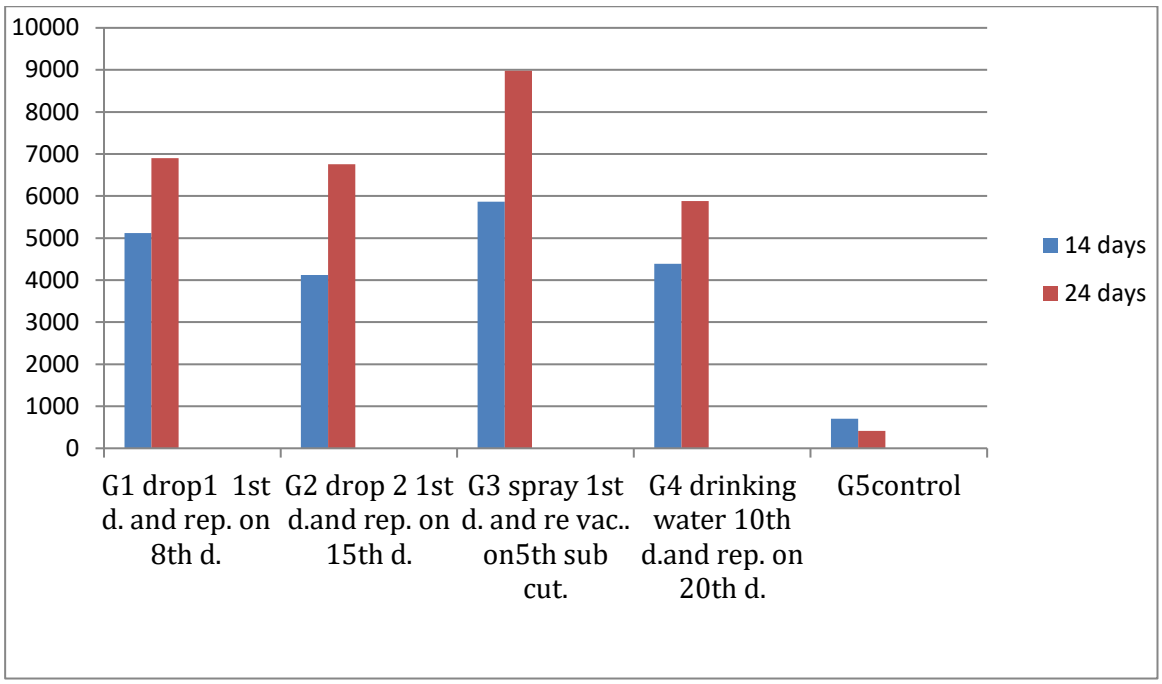

Fig. (1) Volumetric Newcastle antibodies titer means ELISA test measured post vaccination on 14th and 24th day. titer = $10^{log10\ (titer)}$

- **Volumetric antibodies titer means of Newcastle ELISA test measured revealed 24 days post vaccinations .**

Table No. (3) and Figure No. (2) Show compared means of the volumetric Newcastle antibodies titer of which measured of the groups vaccinated

The volumetric antibodies titer of **Oil emulsion vaccine Subcutaneous preceded by a live vaccine by spray method Boosted with live vaccine by drinking water method** increase significantly (P<0.0001) over other vaccine programs on 24th days with value **8980. ± 498.6** .

ND rec .on MD vaccinated group increase significantly (P<0.0001) over two vaccinations dropping methods at different times, injection killed vaccine alone and two vaccinated drinking water method at different times with a value **7211.6 ± 550.2.**

Dropping method in the eye on 1ST day and re-vaccinated on 8th day of the bird's age increase significantly (P<0.0001) over injection killed vaccine alone with value **6898.2 ± 347.1**.

Dropping method in the eye on 1ST day and re-vaccinated on 15th day of the bird's age did not give a significant difference when compared with vaccination injection killed vaccine alone (H9N2 – ND).

Table (3) Volumetric antibodies titer means of Newcastle disease ELISA test measured revealed 24 days post vaccinations .

Vaccine type	ND. Vaccine age	Ab titer (mean ± SE) $10^{log10\ (titer)}$
Spray lasota and sub cut. Injection oil emulsion + DW lasota srian	1st day spray lasota strian+ 5th day sub cut. Injection oil emulsion + 15 day D.W lasota srian	8980. ± 498.6[a]
ND rec .on MD	1st day	7211.6 ± 550.2[b]
Drop 1	1st day and repeated on 8th day of age	6898.2 ±

		347.1^{c}
Drop2	1st day and repeated on 15th day of age	6758.7 ±232.8^{d}
H9N2 –ND (killed)	1st day	6572 ±572.5^{e}
Drinking water	10th day and repeated on 20th day of age	5880.4 ±476.7^{f}
P value		P < 0.0001
LSD (P<0.05)		1383.9

Different letters indicate significant differences at the probability level P < 0.0001.

Negative immune status (ELISA Antibody titer) < 732 , S/P value Immune < 0.5

Positive immune status (ELISA Antibody titer) > 732 , S/P value Immune > 0.5

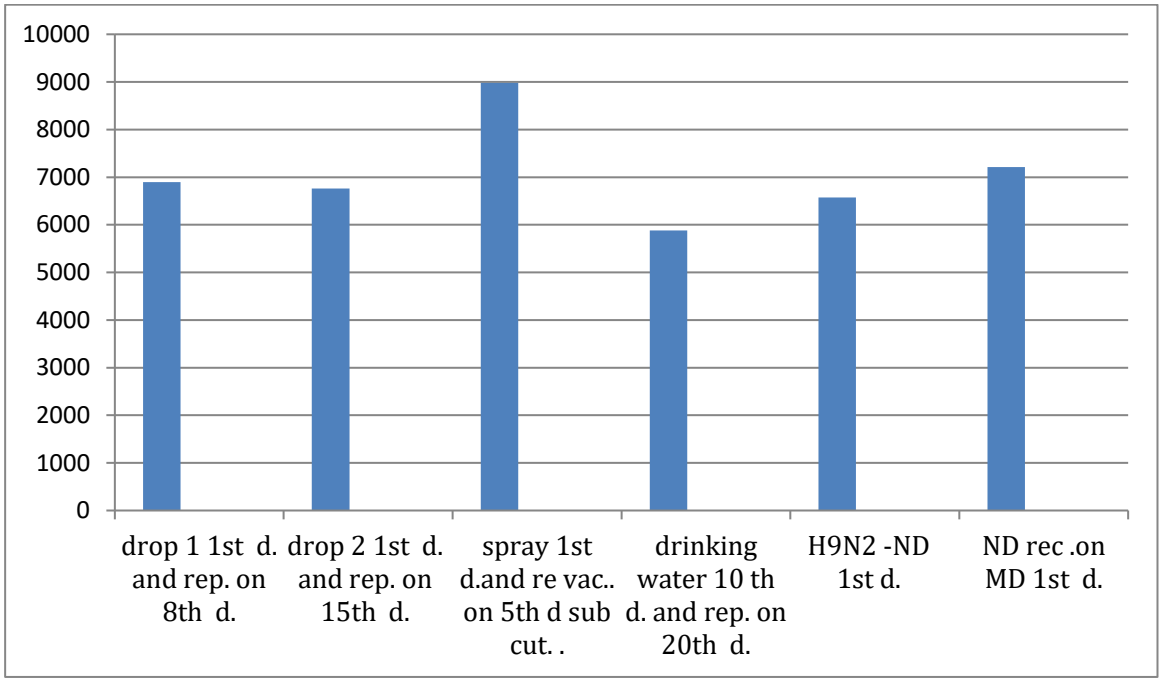

Fig. (2) Compared volumetric Newcastle antibodies titer means ELISA test measured post vaccine types and ways on 24th day. titer = 10$^{log10\ (titer)}$

Discussion

As the Newcastle antigen is present in the tissues, both the live and inactivated vaccines work to support each other in providing protection from Newcastle disease. This is why the vaccination program that uses an oily vaccine before a live attenuated vaccine by spraying and boosted with another live vaccine is more successful than one that uses only live vaccine. It does this by stimulating the local immune system while also causing a slow spread of the killed antigen from the injection site. According to the previously indicated process, the live vaccine serves as a priming agent to stimulate the immune system and induce memory cells for the oil vaccine, while the inactivated vaccine's effectiveness rises with time (Nedeljkovi et al., 2022). (13) After consuming the antigen, the macrophages show a portion of it on their membrane, which prompts B cells to generate antibodies. T-lymphocytes also undergo differentiation and are separated into effector and helper T-cells based on their roles. These cells come into contact with the antigen that is visible on the phagocytic cell membrane. Interferon gamma and cytokine synthesis are also components of the T-cell response.They serve as the predatory arm of T cells (Wei et al., 2024) (14) and heterophil cells by activating phagocytic cells, which increases their capacity to phagocytose and eliminate the pathogen. They can phagocytose and function to make I L-1, I L-6, and I L-8; they are regarded as the initial line of defense for birds' cells.The humeral immune response is triggered by plasma cell-

produced antibodies that circulate in the circulation. The immune cells' surfaces function as receptors once the pathogen enters the body, where some antigens attach and trigger the activation of Th2 T-helper-2 cells (Nedeljkovi et al., 2022)(13).

This led to the filing of agreements with numerous researchers, including Chansiripornchai and Sasipreeyajan (2006) (12), Zamani Moghaddam et al. 2007 (15), Nayak et al. (2009) (16), Wu et al. (2023 (17), Ali et al. (2004) (18), Ellakany et al. (2018) (19), Zeng et al. (2016) (20) ; Wu et al., 2019 (21) and (Lee et al. 2022) (22). Additionally, spray vaccination is a good method with positive benefits, such as early immunity within three days, homogenous immunity, and minimal interference with maternal immunity. These vaccines are administered through the nose, eyes, and mouth, which are the virus's natural entry points.Through inhalation and swallowing, the vaccine promotes mucosal immunity and offers protection (Wegdan et al., 2013)(23).

The lentogenic (Lasota strain) is applied to extremely young chicks without harming the host and can be given through a number of methods, including drinking water, ocular drops, and sprays. We utilized this strain in our investigation because they are also appropriate for secondary immunization in developing and adult birds after an initial vaccination (Alexander and Senne, 2008) (3).

Vaccinated by dropping method (G1) showed good results demonstrating the effectiveness of vaccination with this method when administered on the first day of the bird's age and repeated on the eighth day. This result may be attributed to repeated inoculation by instillation at close intervals in the first group compared to the second group, which received the same vaccination but repeated it on the fifteenth day of the bird's age when measured on the fourteenth and twenty-fourth days of the bird's age. The lacrimal gland (Harderian gland), a crucial component of the immune system, is stimulated by the vaccine drops that land in the eye (Sharma, 2018) (24) and one of the main benefits of this approach is that there is minimal interference between the antibodies produced by the vaccine and the antibodies already in the bloodstream (Kouwenhoven, 1993) (25). NDV antibody titer was increased at 10 d after the first NDV inoculation, and at day 5, 9 and 13 after the second NDV inoculation (Wang et al.2015) (26) .

Other researchers in other fields have found antibodies IgG and IgA in the mucous secretions of the eyes after eye-drop immunization. For example, Davison et al. (2008) found that after eye-drop immunization, Okwor et al. (2013) (28) compared vaccination in the eye with drinking water and found that the instillation method significantly increased the antibodies in the eye compared to the drinking water method, which is in agreement with Neljkovi et al. (2022) (13).

Vaccinated by drinking water method (G4), which was vaccinated on day 10 and repeated ten days later, it did not give good results when measuring the antibody standard on day 14, when compared with the groups vaccinated by other methods. While the antibody standard increased and gave good results when measured on the 24th day of the bird’s age with a value 5880.4. because the antibodies appear in the sera of vaccinated birds within 6 to 10 days after vaccination and reach their highest level two weeks or more later (Alexander et.al,2008) (3). Interferon-gamma is stimulated by this method, and most of the polymeric IgA and IgM found in digestive secretions are then produced locally. This explains why the antibody standard increased slightly four days after vaccination and reached the high level of standard two weeks later, or 24 days of age. Vaccination by spraying, dropping, and drinking water did not significantly differ, according to the researchers (Kibrom Mebrahtu et al., 2018) (29).

Control group (G5), it was significantly lower titer than vaccinated groups when it measured in 14 and 24days from the age of the birds. This is a very natural result of not vaccinating birds ,antibodies standard decrease from on the 14th day to on the 24th day.,antibodies standard decrease from **705.2 ±505.01 on the 14th day to 313.1 ± 560.7 on the 24th day.** Maternal immunity represents the antibodies that are transmitted from hens to newly hatched chicks through egg yolk. The level of acquired antibodies varies from one flock to another and between chicks of the same flock depending on the immune status of the hens (Kitaguchi et.al 2008) (30).One is an equation for the amount of antibodies for hens, and then it begins gradually to decrease after 2-3 days, by (one Log.)

every four and a half days, until it disappears at the age of three weeks.

All vaccinated groups G1,G2,G3,G4 significant increase P<0.0001 in the means of the volumetric antibodies titer on 24th day than the means of the volumetric antibodies titer on 14th day that vaccinated with different ways A distinct immune response is produced depending on the variation in immune stimuli because acquired immunity is developed by the bird's reaction to foreign pathogens or a vaccine that the immune system has not yet recognized, and the bird must activate or activate the mechanisms responsible for dealing with the antigen. quick and efficient immune response, both cellular and humoral, when the bird is exposed to the antigens once more. Immunological memory may be the cause of the substantial rise in antibodies in the second vaccination as opposed to the first, as memory T cells mature and generate antibodies. When the pathogen is later identified, CD4+ T lymphocytes in the same host after primary and secondary infections react rapidly, according to study by Deepali et al. (2020) (31) (Wei et al., 2024) (14). **Recombinant vaccines** (**ND rec. on MD)(G7)** were given excellent result with significant (p<0.0001) immunorespons antibodies titer in early time and as a single dose without any effect of birds health due to have **benefit of** inducing cellular and mucosal responses as well as humoral immunity. Herpes virus of turkey- NDV LaSota have advantage of being safe, efficacious, and less prone to reversion to virulence, recombination, and adverse immunization reactions than live attenuated vaccines and killed vaccine alone (**H9N2-ND**)(G6) This findings agreement with results of researchers such as (Romanutti, 2020) (32) as well as we did not find any immunological interference when inoculated on the first day of the bird's age .

Conclusions

High efficiency of the oil emulsion vaccine administered at 5 days of age, preceded by a live attenuated Lasota vaccine by spray method at one day of age and boosted with a live attenuated Lasota vaccine at 15 days of age and efficiency of the attenuated live vaccine by dropping rout on the first day and repeated it on the eighth day of the bird's age as well as giving the vaccine using drinking water provides protection,when given on the tenth day and repeated on 20th day of the bird's age but at a lower rate than other ways. Recombinant vaccines (ND rec .on MD) have proven to be highly effective in protecting against Newcastle disease than live and Killed vaccine alone as well as killed vaccine (H9N2-ND) provides protection against Newcastle without any effect on the health of vaccinated birds.

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