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EVALUATION OF SOME VACCINATION PROGRAMS AGAINST NEWCASTLE DISEASE VIRUS IN EXPERIMENTALLY CHALLENGED BROILER CHICKENS

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ABSTRACT

The objective of the current study was to evaluate the efficiency of different vaccination programs in protecting experimentally challenged broiler chickens against the VV Strain of Newcastle disease virus. Various live and inactivated NDV vaccines were applied during the trial, including HB1, Colone 124, Lasota, and inactivated vaccine. Broiler chicks were divided into 5 groups: the first 3 groups underwent different vaccination programs against NDV, while the other 2 groups (groups 4 and 5) were kept without vaccination to be the control groups. The challenge was done at day 28th of age via intranasal administration of NDV velogenic GVII (NDV/CK/Egypt/F33/2021). Ab titers were determined on days 1,7,14,21,28 and 35 of the experiment. The results of the shedding titers of NDV indicated that the lowest shedding titer was observed in G3, G2 (vaccinated with live and inactivated vaccine), followed on days 3, 5, and 7 post-challenge on the 28th, compared to G1 (vaccinated with live vaccine only). Also, no mortalities (100% protection rate) were recorded in group (3) vaccinated with both live and double shots of killed NDV vaccines, compared to low mortality rates recorded in group (2) vaccinated with live vaccines and one shot of inactivated vaccine and those vaccinated with live vaccines only. The recorded results indicated that ND vaccination programs utilizing both live and double shots of inactivated vaccines were more effective than those depending on a single shot of inactivated vaccine combined with live vaccines, as well as more effective than programs consisting only of live vaccines.

Keywords: Vaccination Programs, Live, and Inactivated Vaccines, AB titers, Shedding.

INTRODUCTION

Poultry production is one of the animal businesses with the fastest global growth rates, along with swine production. Global demand for poultry products is rising continuously, with an average of 2% every year in consumption (FAO 2015). Poultry diseases provide two primary problems in Egypt and developing countries. The first threat comes from the fatal species-specific chicken diseases like Newcastle Disease Virus (NDV), Infectious Bursal Disease Virus (IBDV), and Mycoplasma spp., which not only reduces the efficiency, growth rate, and

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expansion of the poultry industry, but also raises its economic cost.

Newcastle disease (ND) is one of the most fatal diseases affecting poultry all over the world, and can cause death up to 80% of backyard chickens in Africa every year (Cappelle *et al.,* 2015). ND is caused by avian paramyxovirus-1 (APMV-1), one of the antigenically distinct avian paramyxoviruses 1 – 11, genus Avulavirus, family Paramyxoviridae, and order Mononegavirales (ICTV, 2012). The International Committee on Taxonomy of Viruses recently changed the nomenclature of all avian paramyxoviruses, including NDV of the family Paramyxoviridae, and gave them the new name avian Avulavirus (AAvV) (Wajid *et al.,* 2017). Newcastle disease viruses are single-stranded, non-segmented, negative-sense RNA viruses with one of three genome sizes that encode for at least six structural proteins (Miller and Koch, 2013). Newcastle disease virus has also been divided into five pathotypes, which are identified as follows: a) viscerotropic velogenic; b) neurotropic velogenic; c) mesogenic; d) lentogenic or respiratory; and e) asymptomatic, depending on the clinical signs observed in infected chickens (CFSPH, 2016).

Clinical indications that were noted in commercial broiler chickens were paresis, green diarrhea, severe depression, and death 48–72 hours after the sickness started. Numerous investigations have documented additional symptoms, such as severe conjunctivitis, face edema, and birds standing motionless with drooping wings. Furthermore, layer flocks showed a 50% decline in egg production (Mansour *et al.,* 2021). Rales, coughing, sneezing, and gasping are respiratory tract symptoms. Tremors, paralyzed legs and wings, twisted necks, circling, clonic spasms, and even total paralysis are neurological system symptoms. Additional common symptoms include greenish diarrhoea, inappetence and depression, a partial or total decrease in egg production, and a rise in the number of eggs with abnormalities (Abdisa and Tagesu, 2017).

Vaccines are frequently used in commercial chicken production to reduce and/or control field problems caused by bacteria, viruses, or protozoa. Moreover, vaccines are given to breeder hens to maximize the amount of maternal immunity transferred to hatchling chicks (Murtada, 2017). In addition to effective management and biosecurity procedures, vaccination should be used. For backyard or village poultry to survive, vaccination is required in various parts of the world where vNDV is endemic (Suarez *et al.,* 2020). Inactivated vaccinations generate a significant number of antibodies against NDV and offer a strong defense against the highly pathogenic virus (Alexander *et al.,* 2004). Live vaccines provide both mucosal and humoral immunity and can be administered using mass application techniques (Dimitrov *et al.,* 2017). It is not necessary to vaccinate every bird individually as in inactivated vaccine. The random use of intensive vaccines, frequent mutations, and the introduction of novel pathotypes of NDV may be the cause of many NDV outbreaks. Egypt has documented cases of NDV genotypes II and VII (Naguib *et al.,* 2022).

Aim of this study:

In Egypt, where ND is endemic, vaccination is a routine preventive measure from the first day of chick age. We advise strengthening the vaccine program, since ND outbreaks are still known to happen despite this preventive measure. Thus, the current study's objective was to evaluate the efficiency of different vaccination programs in protecting experimentally challenged broiler chicken against VV strain of Newcastle disease virus (NDV), determination of virus shedding of different groups indicating the extent and period of infectiousness by qRT-PCR, comparison of Abs titer using Hemagglutination Inhibition (HI) test, and

comparison of body weight and feed conversion rates between different groups.

MATERIALS AND METHODS

1. Commercial Broiler Chicks

A total of 125 Arbor Acres broiler chicks, with an average body weight of 40g, were acquired from Elkasaby Poultry Company. Biosecurity measures were considered, floor rearing on a deep litter of 7 cm thickness with a stocking density of 10 birds / m2 was applied to the birds in separated, disinfected, and isolated experimental areas. The birds were fed a commercial fattening feed made by El-salam company, which consisted of three different types of food: 600–700 g starter diet per bird, which had 23% protein; 1500 g grower diet per bird, which had 21% protein; and 1000 g finisher diet per bird, which had 19% protein. Before grouping, maternal immunity was assessed in five one-day-old chicks that were chosen randomly and slaughtered for blood collection. Every week until the conclusion of the trial, each group's ultimate body weight and feed conversion rate were measured.

2. Newcastle disease challenge virus:

The vNDV challenge virus NDV/CK/ Egypt/F33/2021 (acc. no. MZ409479.1) belongs to genotype VII and was kindly provided by Reference Laboratory for Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt. The virus challenge dose equals 6-Log-10 EID50 given 0.1 ml/bird via the intranasal route (OIE, 2012).

3. IBD vaccine:

The vaccine used was Gumbokal IM SPF, the company was HYPER VET, the dose was1000d/Vail, and the strain used/dose is IM strain VMG 91 ≥ 103.5 TCID50.

4. NDV Vaccines:

As shown in Table 1, various live and inactivated NDV vaccines were utilized during the trial. Inactivated vaccines were administered by subcutaneous injection in the neck skin fold, while live vaccines were administered by ocular instillation.

Table 1: An explanation of the NDV vaccines utilized in the experiment's several vaccination protocols

Vaccine used	Company	Dose	Strain used/dose
POLIMUN ND HITCHNER B1	BioTestLab (Ukraine)	1000 d/vail	Hitchner B1 ≥ $10^{6,0}$ EID_{50} per dose
POLIMUN ND CLON 124	BioTestLab (Ukraine)	1000 d/vail	La Sota Clon DK-124 ≥ $10^{6,0}$ $EID_{50.}$
POLIMUN LA-SOTA	BioTestLab (Ukraine)	1000 d/vail	La-Sota"$10^{6,0}$ EID_{50} per dose
POLIMUN ND INAC	BioTestLab (Ukraine)	1000 d/vail 0.1cc	La Sota ≥ $10^{9,0}$ lg EID_{50}

5. NDV reference antigens:

ND Lasota vaccinal strain was diluted to 4 HAU to be used as HA antigen in hemagglutination inhibition (HI) titration of ND antibody obtained from BioTestLab. Dilution was performed by PBS.

6. Serum samples:

Every week, a random and individual blood sample was taken in a vacuum gel and clot activator tube directly from the wing vein of the experimental chicks. The serum sample was separated in a dry Eppendorf container at -20 ^{o}C till serological analysis was performed to detect NDV antibodies.

7. Chicken erythrocytes suspensions:

Red blood corpuscles (RBCs) were collected from chickens from wing vein punctures in tubes containing Ethylenediaminetetraacetic acid (EDTA) as

an anticoagulant. The RBCs were washed with physiological saline by centrifugation at 3000 rpm/10 minutes each time three times or till a clear supernatant was obtained. For the HI test, the washed packed RBCs were diluted with 1% saline to evaluate the human immune response to the ND vaccine (Ayoub *et al.,* 2019).

Experiment design:

Chicks were divided into 5 groups. All groups received a preventive program from day one till the end of the experiment . Groups from 1 to 3 contained 20 birds in each one and underwent different vaccination programs against NDV (Table ٢), while groups 4 and 5 were kept without vaccination to be the control groups, group 4 contained 30 birds and acted as a control +ve group (challenged put not vaccinated) and group 5 contained 30 birds and acted as a control negative one (not challenged and not vaccinated).

Table 2: Description of vaccination programs applied to the different experimental groups

Age groups	Day 1	Day 10	Day 14	Day 18	Day 28
G1	HB1	Clon 124	Gumbokal	Lasota	Challenge by NDV (10^6 ELD_{50}) intranasal
G2	HB1	Clon 124 / Killed vaccine	Gumbokal	Lasota	
G3	HB1 / Killed vaccine	Clon 124 / Killed vaccine	Gumbokal	Lasota	
G4	No vaccination program				
G5	No vaccination program			No challenge	

Antibodies titers were determined at day one in 5 randomly selected chicks for maternal antibody and at days 7,14,21,28, and 35 of the experiment in 5 randomly selected chicks from each group. Before the challenge on day 28, each group from 1 to 3 was subdivided into 2 groups (one for challenging and the other not and used for blood collection on day 35). A challenge was done on day 28 to subgroups from 1 to 3 via intranasal administration of NDV (10^6 EID_{50}) velogenic GVII (NDV/CK/ Egypt/F33/2021). Finally, shedding of NDV was detected in oropharyngeal (tracheal) and cloacal swabs by using qPCR at days 3, 5, and 7 post-challenge. Chicken blood was collected by slaughtering 5 chicks one day old (1 ml) and by puncture of wing vein at 7,14,21,28, and 35 days old (2 ml) and kept in slope position in a vacuum gel and clot activator tube at 37 °C for one hour, then at 4°C overnight. Sera was then separated by centrifugation at 3000 rpm/10 minutes and stored in an Eppendorf at -20 °C till tested.

Assessment Parameters:

1. Shedding Titer:

Virus shedding will be determined in Oropharyngeal (tracheal) and cloacal swabs by using qRT-PCR. Using the method described by Wise, Suarez, *et al.,* (2004).

2. Humoral Immunity:

Expressed by titration of Abs by HI test at age 7,14,21,28,35 of age. Using the method described by Ayoub *et al.,* (2019).

3. Mortality Rate:

$$\text{Mortality rate} = \frac{\text{Number of dead birds}}{\text{total number of birds in each group}} \times 100$$

4. Feed conversion ratio:

FCR for all weeks in the experiment was estimated, according to Fritz *et al.,* (1969) as follows:

$$\text{FCR} = \frac{\text{Feed intake (g) in a given period}}{\text{Body weight gain (g) in the same period}} \times 100$$

Statistical analysis:
It was made using a repeated measure of one-way ANOVA test, Partial eta squared, the Bonferroni test, Z test, and Pearson correlation coefficient (r). All these tests were used to examine the significant differences in the detection rate of antibodies among different groups, shedding titer of virus studied, FCR, and mortality rate. A probability (p) value ($P < 0.05$) was considered statistically significant**.** Interpret values for Partial eta squared (0.14 or higher) were considered a large effect size.

RESULTS

Table 3: Mortality rate of chicken in different experimental groups after 7d post-challenge

Experimental Groups	DPC	Mortality		Protection			
		No.	%	No.	%	Z	P
Group 1	1 bird at 31th day	1	10%	9	90%	٠.١،٢	.230140
Group 2	1 bird at 31th day	1	10%	9	90%	1.8	0.07186
Group 3		0	0%	10	100%	NA	
Group 4 (control +ve)	4 birds at 31th day	15	100%	0	0		
	7 birds at 33th day						
	4 birds at 37th day						
Group 5 (control -ve)	Not challenged						

1. Laboratory detection and identification:

1.1. Serological test:

Table 4: HI titers of one-day-old chicks, (maternal immunity):

Days of study	Titer									Mean±SD	Sphericity Assumed groups	
	0	1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
1d							2	1	2	7±1	0.000	0.918

Table 5: HI titers of 7-day-old chicks of experimental groups 1,2,3,4,5.

Days of study	Study groups	Titer								Mean±SD	Sphericity Assumed groups	
		1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
7d	G1			1	2	2				4.20±0.83	0.000	0.918
	G2				3	2				4.40±0.54		
	G3				3	2				4.40±0.54		
	G4			3	2					3.40±0.54		
	G5			2	2	1				3.80±0.83		

Table 6: HI titers of 14-day-old chicks of experimental group 1,2,3,4,5.

Days of study	Study groups	Titer									Mean±SD	Sphericity Assumed groups	
		0	1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
14d	G1				1	2	1	1			4.40±1.14	0.000	0.918
	G2					1	3	1			5.00±0.707		
	G3					1	2	2			5.20±0.83		
	G4			2	3						2.60±0.54		
	G5		2	2	1						1.80±0.83		

Table 7: HI titers at 21-day-old chicks of experimental group 1,2,3,4,5:

Days of study	Study groups	Titer									Mean±SD	Sphericity Assumed groups	
		0	1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
21d	G1					1	2	2			5.20±0.83	0.000	0.918
	G2					1	1	2	1		5.60±1.14		
	G3						1	3	1		6.00±0.707		
	G4	2	1	2							1.00±1.00		
	G5	1	3	1							1.00±0.707		

Table 8: HI titers at 28-day-old chicks of experimental groups 1,2,3,4,5.

Days of study	Study groups	Titer									Mean±SD	Sphericity Assumed groups	
		0	1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
28d	G1					1	1	3			5.40±0.89	0.000	0.918
	G2					1	1	2	1		5.60±1.14		
	G3						1	2	2		6.20±0.83		
	G4	3	2								0.40±0.54		
	G5	3	2								0.40±0.54		

Table 9: HI titers at 35-day-old chicks of experimental groups 1,2,3,4,5:

Days of study	Study groups	Titer									Mean±SD	Sphericity Assumed groups	
		0	1	2	3	4	5	6	7	8		Sig.	Partial Eta Squared
35d	G1				1	2	2				4.20±0.83	0.00	0.918
	G2					1	3	1			5.00±0.707		
	G3						1	3	1		6.00±0.707		
	G4	5									0.00±0.00		
	G5	5									0.00±0.00		

Detection of the NDV shedding in the tracheal and cloacal swabs after challenge by Real time RT-PCR

Five tracheal and cloacal swaps were collected from each group at 3,5,7 DPC to detect virus shedding using Real time RT-PCR.

DISCUSSION

The results of the current study concerning clinical signs observed of NDV experimental infection through intranasal routes included depression, anorexia, weight loss, watery greenish diarrhea, paralysis, and anomalies of the legs in G1, G2, and G4 that were similar to those observed by (Susta *et al.,* 2011; Ratih *et al.,* 2017; El-Morshidy *et al.,* 2021). Greenish watery diarrhea was recorded in challenged groups 1 and 4, which may suggest higher viral local replication through the GIT and this result was agreed with (Abd El Aziz *et al.,* 2016). None-theless, all groups exhibited more severe respiratory symptoms, such as abnormal respiratory sounds, and difficulty breathing (Abd El Aziz *et al.,* 2016; Mariappan *et al.,* 2018; Moharam *et al.,* 2019). All inoculated groups displayed nasal discharge and a mouth cavity filled with mucous secretion due to intranasal viral inoculation, which resulted in abundant mucus secretion in the nasal cavity and stasis of GIT movement (Miller *et al.,* 2013).

Table 10: ND titer of tracheal and cloacal shedding at 3,5,7 DPC.

	Groups	DPC	TS	CS	Mean± SD
Titer	group 1	3-day post challenge	3.463 X 10^5	7.725 X 10^5	559400±301368.91
		5-day post challenge	1.235 X 10^5	1.460 X 10^6	791750±945048.21
		7-day post challenge	0	0	0.00±0.000
	group 2	3-day post challenge	5.733 X 10^4	3.842 X 10^5	220765±231131.99
		5-day post challenge	5.276 X 10^4	2.400 X 10^5	146380±132398.67
		7-day post challenge	0	0	0.00±0.000
	group 3	3-day post challenge	7.147 X 10^3	5.976 X 10^4	33453.50±37203.00
		5-day post challenge	4.818 X 10^3	2.448 X 10^4	14649.00±13903.13
		7-day post challenge	0	0	0.00±0.00
	group 4	3-day post challenge	1.619 X 10^6	3.168 X 10^6	2393500±1095308.4
		5-day post challenge	2.977 X 10^6	8.697 X 10^6	5837000±4044650.7
		7-day post challenge	1.952 X 10^6	5.863 X 10^6	3907500±2765494.6

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

*. The mean difference is significant at the 0.05 level.

Table 11: Influence of various NDV vaccination programs on broiler chicken productivity across various experimental groups

experimental groups	final body weight at 35 days old (g)	total feed consumption (g)	Feed conversion rate (FCR)
Group 1	2001.39	3510	1.75
Group 2	2133.5	3560	1.67
Group 3	2204.65	3595	1.63
Group 4 (+ve)		all birds died	
Group 5 (-ve)	2297.64	3600	1.57
R		0.965541	
P		0.000	
R			0.999
P			0.000

Regarding the mortality rate of broiler chickens following the vNDV challenge (Table 3), the non-vaccinated challenged group (G4), where no vaccination program occurred, had the greatest rate of mortality (100%). In contrast, groups (G3) did not experience any mortalities (100% protection rate). Furthermore, minimal mortality rates were noted in G1 and G2. On the third day post-challenge, there were six early deaths noted in birds of groups 1, 2 and 4, which were inoculated with the virus via the intranasal route. On the third day post-challenge, there were six early deaths noted in birds of groups 1, 2, and group 4 which were inoculated with the virus via the intranasal route, and this disagreed with (Wang *et al.,* 2012), who noticed that early deaths on the third-day post inoculated were recorded in birds of the mixed intraocular–intranasal inoculated group. Fentie *et al.* (2014) proved that the detection of the challenge virus in most vaccinated birds confirmed that the tested vaccination protocols cannot completely protect birds from viral infection, replication, and shedding, and vaccinated–infected birds can act as a source of infection for susceptible flocks. The high mortality observed in unvaccinated birds

and their contacts confirmed the virulence of the challenge virus and indicated that this field virus strain can easily spread in an unvaccinated poultry population and cause major outbreaks. Progressive vaccinations supported by biosecurity measures should therefore be applied to control the disease and introduction of the virus to poultry farms Fentie *et al.,* (2014).

In the present study, postmortem findings included redness observed in the carcasses of dead birds during postmortem examination, catarrhal exudate in the trachea, ulceration of cecal tonsils, petechial haemorrhage on the tip of the proventriculus gland, elliptical ulcer of the intestine and enlargement and congestion in the kidney and liver in different groups and this result agreed with (El-Morshidy *et al.,* 2021).

Our findings demonstrated that the Abs titers (Mean±SD) for 14-day-old chicks revealed a significant difference at ($P < 0.05$) between the groups according to statistical analysis. The group (3) that received the vaccines at days 1, 10, and 18 with a live+ double shot of inactivated vaccine, had the highest antibodies titers (Mean±SD 5.20±0.83). This group was followed by those who received the vaccines at days 1, 10, and 18 with live+ inactivated vaccines, respectively (G2) (Mean±SD 5.00±0.707). On the other hand, the non-vaccinated positive and negative control groups had the lowest Abs titers (Mean±SD 2.60±0.54 and 1.80±0.83, respectively) (G4, G5). At 21 days, the Mean±SD showed a significant difference between the groups at ($P < 0.05$) according to statistical analysis. Once again, the vaccinated groups that received both live and inactivated vaccines had the highest Ab titers (Mean±SD 5.60±1.14 and 6.00±0.707) (G2 and G3), in contrast to the vaccinated group that received only the live vaccine (Mean±SD 5.20±0.83) (G1). Compared to chicks that were 7 and 14 days old, the titers in the control groups were lower and displayed the lowest Ab levels. Statistical analysis at 28 days revealed a significant difference in the Mean±SD between the groups at ($P<0.05$). The groups that received a double shot of inactivated vaccines and live vaccinations continued to have the highest Ab titers across all other groups. In comparison to groups vaccinated with live vaccine only (Mean±SD 5.40±0.89) (G1), levels of titers were higher than their level at 14 days, indicating a higher protection level (Mean±SD 6.20±0.8) (Table 10). Our results agreed with the findings of Vrdoljak *et al.* (2018) who recorded that vaccination of 1-7 days old broilers with live attenuated ND vaccine provides significant protection against field vNDV, despite the presence of MDA. Kapczynski *et al.* (2006), who determined that there was a positive correlation between reduced viral shedding and the existence of hemagglutination-inhibiting antibody titers during challenge. On the other hand, the immunological response to NDV vaccines at 35 days old, or 7 days after the challenge (G3) that received both live and double shots of inactivated vaccines, exhibited the highest Abs titers among all groups. However, the levels of titers were lower than that at 28 days of age (Mean±SD 6.00±0.707) compared to groups that received live vaccine alone and that received both live vaccine and one shot of inactivated vaccine (Mean±SD 4.20±0.83 and 5.00±0.707) (G1, G2). Additionally, it was noted that the control positive group's (G4) non-vaccinated, NDV-challenged chicks and the control negative group's (G5) non-vaccinated and non-challenged chicks were zero in antibody titer. The administration of double shots of inactivated NDV vaccines and live NDV vaccines in (G3), produced significant high levels of humoral antibodies specific to NDV and completely protected the chickens against death following an intranasal challenge with VV NDV at age 28. Additionally, the vaccine significantly reduced the amount of virus shedding into the environment, which in turn reduced the number of secondary infected birds.

Although vaccination approaches are comparatively efficient in the prevention of severe illness and deaths of infected birds, some of them may fail to prevent either infection or virus shedding (Mansour *et al.,* 2021).

The findings of Ellakany *et al.* (2019), who discovered that no vaccine could prevent shedding and that the protection percentage of commercially available live and inactivated vaccines gave varying levels of protection against mortalities and viral shedding, confirmed the obtained results. The combination of inactivated NDV vaccine and LaSota were found to be the most effective in preventing morbidity and mortality (100% and 93%, respectively), while only Hitchner B1 priming for inactivated vaccine protected the birds from viral shedding at 5- and 7-days post-infection.

The tracheal and cloacal shedding titer from challenged broiler chickens by NDV at the 3-day post-challenge was recorded in Table 10. The results of the Bonferroni Test analysis revealed a significant difference in the NDV shedding titers of the various experimental groups. G3 received vaccinations at days 1, 10 and 18 with live+ double shot inactivated vaccine, which had the lowest shedding titer. In contrast, the control positive group (G4), which was not vaccinated and was challenged with NDV, had the greatest shedding titer, followed by G1, which received the live only at days 1,10, and 18, respectively then G2 which received live+ inactivated vaccine at days 1,10 and 18, respectively. Like most vaccines, NDV vaccines do not prevent vaccinated birds from becoming infected with a vNDV and subsequently shedding the virus, however, most vaccines will significantly decrease the amount of virus shed in saliva and feces compared to non-vaccinated birds Hu *et al.* (2009).

Nonetheless, compared to nonvaccinated birds, most vaccinations will considerably reduce the virus-shedding amount in the saliva and feces (Miller *et al.,* 2009).
As indicated by Table ١٠, it was found that the experimental groups (G3) at 5dpc exhibited the lowest NDV shedding titers, indicating an effective vaccination program. On the other hand, the control positive group (G4), which was not vaccinated and was challenged by NDV, had the greatest shedding titer (5837000±4044650.788), which was followed by G1 (791750±945048.21), then G2 (146380±132398.67). The results found agreed with the results of Kapczynski and King (2005), who concluded that live and inactivated vaccination considerably decreased the incidence and viral titers shed by chickens and protected against morbidity and mortality, but they did not stop infection or virus shedding and agreed with the result of (Ayoub *et al.,* 2019) who concluded that application of ND vaccination programs containing both live and double inactivated vaccines (either GII or GVII) was found to be more effective than those depending on one shot of inactivated vaccine (either GII or GVII) plus live vaccines and more effective than program including live vaccines only.

Our study illustrates the challenging NDV's tracheal and cloacal shedding from broiler chickens at the 7DPC. It was noted that the experimental G1, G2, and G3 did not exhibit any apparent NDV shedding titers, indicating an effective vaccination program and this result disagreed with the result of (Ayoub *et al.,* 2019) who concluded that the group that received live vaccine only still shed high titer of the virus at 7th day post-challenge. On the other hand, the highest shedding titer (3907500±2765494.621) was obtained in G4. According to these results, a combination of live and inactivated NDV vaccines may significantly reduce viral shedding while also preventing mortality and morbidity. This may be explained by the extremely high antibody levels that the suggested vaccination program produced

in addition to the effectiveness of vaccinations administered in experimental settings (flock immunity) (Van Boven *et al.,* 2008). However, it is important to remember that multiple factors may work together to reduce vaccination effectiveness in the field thus making the antibody specificity more important. (Miller *et al.,* 2013). The observed results regarding protection agreed with the findings of (Saad *et al.,* 2017), who investigated the capacity of heterologous antibodies produced by commercially available vaccines based on lentogenic strains, to efficiently reduce viral shedding and showed that the provided vaccination program (Clone 30 with killed NDV vaccine) produced enough heterologous antibody levels to adequately protect birds against disease and mortality. Since there are currently no vaccinations that can produce homologous antibodies to the viruses that are in circulation, reducing the spread of virus shedding is thought to depend critically on increased antibody levels, which are based on the timely and efficient application of the vaccination program.

Regarding to the impact of various NDV vaccination programs on the broiler chickens' productive performance throughout the experiment. Significant differences in the estimated FCR of each experimental group were shown by statistical analysis. The results showed that the combination of live and inactivated vaccines in immunization against NDV did not adversely affect the feed conversion ratio. Specifically, FCR was higher in groups vaccinated with live and inactivated vaccines (G2, G3) compared to those vaccinated with live vaccines only (G1) (1.75). The results obtained were in line with those of Alexander and Senne (2003), who stated that vaccination with live vaccines may cause disease occurrence and reduce growth rates of vaccinated birds. Although Chansiripornchai and Sasipreeyajan (2006), who discovered that the body weight gain, feed intake, and feed conversion ratio (FCR) of the group vaccinated subcutaneously at 1 day old with inactivated oil adjuvant vaccine (IOAV) and live vaccine were significantly better than those of the group vaccinated subcutaneously at 1 day old with IOAV in combination with live vaccine during 1–42 days old.

Our study illustrated that there was a significant difference between vaccinated groups (G1, G2, G3) (3510, 3560, 3595), respectively, and the non-vaccinated, non-challenged group (G5) (3600) in the total feed consumption. These results disagreed with (Martinez *et al.,* 2018), who studied the evaluation of the effect of live LaSota Newcastle disease virus vaccine as primary immunization on immune development in broilers. They concluded the vaccine did not affect cumulative feed intake, because there were no significant differences ($P>0.05$) between vaccinated and unvaccinated birds at days 14, 28, and 42. These results agreed with (Ayoub *et al.,* 2019), who studied the Evaluation of Some Field Vaccination Programs Recommended for the Protection of Broiler Chicken against New Newcastle disease. They discovered that feed intake in groups that received both live and inactivated vaccines was higher than in those who received the live vaccine only.

CONCLUSIONS

The combination of the live and inactivated ND vaccine significantly improves protection in broiler chickens, compared to using the live vaccine alone. In the management of vNDV, the combination of live and inactivated vaccination reduced not only the mortality of challenged chickens, but also the quantity of virus shed through the trachea and cloaca.

REFERENCES

Abd El Aziz, M.; El-Hamid, A.; Ellkany, H.; Nasef, S.; Nasr, S. and El Bestawy, A. (2016): Biological and molecular characterization of Newcastle disease virus circulating in chicken flocks, Egypt, during 2014-2015. *Zagazig Veterinary Journal*, *44*(1), 9-20.

Abdisa, T. and Tagesu, T. (2017): Review on Newcastle disease of poultry and its public health importance. *J. Vet. Sci. Technol*, *8*(3), 441.

Alexander, D.J. and Senne, D. (2003): Newcastle disease. *Diseases of Poultry*. 11:64-87.

Alexander, D.J.; Bell, J.G. and Alders, R.G. (2004): A technology review: Newcastle disease, with special emphasis on its effect on village chickens.

Ayoub, M.A.; Elfeil, W.K.; El Boraey, D.; Hammam, H. and Nossair, M.A. (2019): Evaluation of some vaccination programs in protection of experimentally challenged broiler chicken against Newcastle disease virus. *Am. J. Anim. Vet. Sci*, *14*(3), 197-206.

Cappelle, J.; Caron, A.; De Almeida, R.S.; Gil, P.; Pedrono, M.; Mundava, J. and Gaidet, N. (2015): Empirical analysis suggests continuous and homogeneous circulation of Newcastle disease virus in a wide range of wild bird species in Africa. *Epidemiology & Infection*, *143*(6), 1292-1303.

Center for food security and public health (CFSPH) (2016): IOWA State University College of Veterinary Medicine.

Chansiripornchai, N., and Sasipreeyajan, J. (2006). Efficacy of live B1 or Ulster 2C Newcastle disease vaccines simultaneously vaccinated with inactivated oil adjuvant vaccine for protection of Newcastle disease virus in broiler chickens. *Acta Veterinaria Scandinavica*, *48*, 1-4.

Dimitrov, K.M.; Afonso, C.L.; Yu, Q. and Miller, P.J. (2017): Newcastle disease vaccines—A solved problem or a continuous challenge?. *Veterinary microbiology*, *206*, 126-136.

Ellakany, H.; El-Hamid, A.; Nasef, S.; Abdel Aziz, M.; Gado, A. and Zedan, R. (2019): Evaluation of the protection of commercial live and inactivated NDV vaccines against Newcastle virus genotype VIId circulating in the field. *Damanhour Journal of Veterinary Sciences*, *1*(1), 17-20.

El-Morshidy, Y.; Abdo, W.; Elmahallawy, E.K.; Abd EL-Dayem, G.A.; El-Sawak, A.; El-Habashi, N. and Abou Asa, S. (2021): Pathogenesis of velogenic genotype VII. 1.1 Newcastle disease virus isolated from chicken in Egypt via different inoculation routes: molecular, histopathological, and immunohistochemical study. *Animals*, *11*(12), 3567.

FAO (2015): OECD-FAO Agricultural Outlook, 2015-2024, OECD.

Fentie, T.; Dadi, K.; Kassa, T.; Sahle, M. and Cattoli, G. (2014): Effect of vaccination on transmission characteristics of highly virulent Newcastle disease virus in experimentally infected chickens. *Avian Pathology*, *43*(5), 420-426.

Fritz, J.C.; Roberts, T.; Boehne, J.W. & Hove, E.L. (1969): Factors affecting the chick's requirement for phosphorus. *Poultry Science*, *48*(1), 307-320.

Hu, S.; Ma, H.; Wu, Y.; Liu, W.; Wang, X.; Liu, Y. & Liu, X. (2009): A vaccine candidate of attenuated genotype VII Newcastle disease virus generated by reverse genetics. *Vaccine*, *27*(6), 904-910.

ICTV (2012): A. King, E. Lefkowitz, M.I. Adams and E. B. Carstens. Virus

taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Academic Press, London, United Kingdom.

Kapczynski, D.R. and King, D.J. (2005): Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine*, *23*(26), 3424-3433.

Kapczynski, D.R.; Wise, M.G. and King, D.J. (2006): Susceptibility and protection of naïve and vaccinated racing pigeons (Columbia livia) against exotic Newcastle disease virus from the California 2002–2003 outbreak. Avian Diseases, 50(3), 336-341.

Mansour, S.M.; ElBakrey, R.M.; Mohamed, F.F.; Hamouda, E.E.; Abdallah, M.S.; Elbestawy, A.R. and Eid, A.A. (2021): Avian paramyxovirus type 1 in Egypt: epidemiology, evolutionary perspective, and vaccine approach. *Frontiers in veterinary science*, *8*, 647462.

Mariappan, A.K.; Munusamy, P.; Kumar, D.; Latheef, S.K.; Singh, S.D.; Singh, R. and Dhama, K. (2018): Pathological and molecular investigation of velogenic viscerotropic Newcastle disease outbreak in a vaccinated chicken flocks. *Virusdisease*, *29*, 180-191.

Martinez, J.C.S.; Chou, W.K.; Berghman, L.R. and Carey, J.B. (2018): Evaluation of the effect of live LaSota Newcastle disease virus vaccine as primary immunization on immune development in broilers. *Poultry science*, *97*(2), 455-462.

Miller, P.J.; Afonso, C.L.; El Attrache, J.; Dorsey, K.M.; Courtney, S.C.; Guo, Z. and Kapczynski, D.R. (2013): Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Developmental & Comparative Immunology*, *41*(4), 505-513.

Miller, P.J. and Koch, G. (2013): Newcastle disease, p 89–138. *Diseases of poultry, 13th ed. Wiley-Blackwell, Hoboken, NJ*.

Miller, P.J.; Estevez, C.; Yu, Q.; Suarez, D.L. and King D.J. (2009): Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild-type and recombinant viruses. Avian. Dis. 53: 39–49.

Moharam, I.; Razik, A.A.E.; Sultan, H.; Ghezlan, M.; Meseko, C., Franzke, K. and Grund, C. (2019): Investigation of suspected Newcastle disease (ND) outbreaks in Egypt uncovers a high virus velogenic ND virus burden in small-scale holdings and the presence of multiple pathogens. *Avian Pathology*, *48*(5), 406-415.

Murtada, M.R. (2017): Development of a vaccine against Newcastle Disease and antimicrobials for Salmonella serovars (Doctoral dissertation).

Naguib, M.M.; Höper, D.; Elkady, M.F.; Afifi, M.A. Erfan, A.; Abozeid, H.H. and Grund, C. (2022): Comparison of genomic and antigenic properties of Newcastle Disease virus genotypes II, XXI and VII from Egypt do not point to antigenic drift as selection marker. *Transboundary and Emerging Diseases*, *69*(2), 849-863.

OIE, (2012): Newcastle disease. Chapter 2.3.14. Terrestrial Manual 7th Edition of Standards for Diagnostic Tests and Vaccines. 1: 555-574. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.14_NEWC ASTLE_DIS.pdf.

Ratih, D.; Handharyani, E. & Setiyaningsih, S. (2017): Pathology and immunohistochemistry study of

Newcastle disease field case in chicken in Indonesia. *Veterinary world*, *10*(9), 1066.

Saad, A.M.; Samy, A.; Soliman, M.A.; Arafa, A.; Zanaty, A.; Hassan, M.K. and Hussein, A.H. (2017): Genotypic and pathogenic characterization of genotype VII Newcastle disease viruses isolated from commercial farms in Egypt and evaluation of heterologous antibody responses. *Archives of Virology*, *162*(7), 1985-1994.

Suarez, D.L.; Miller, P.J.; Koch, G.; Mundt, E. and Rautenschlein, S. (2020): Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. *Diseases of poultry*, 109-166.

Susta, L.; Miller, P.J.; Afonso, C.L. and Brown, C.C. (2011): Clinicopathological characterization in poultry of three strains of Newcastle disease virus isolated from recent outbreaks. *Veterinary pathology*, *48*(2), 349-360.

Van Boven, M.; Bouma, A.; Fabri, T.H.; Katsma, E.; Hartog, L. and Koch, G. (2008): Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian pathology*, *37*(1), 1-5.

Vrdoljak Anto, Máté Halas and Tamás Süli (2018): Efficacy of Live Attenuated Vaccines against Newcastle Disease in Commercial Broilers. *Journal of Veterinary Medicine and Research,* 5(2): 1123.

Wajid, A.; Dimitrov, K.M.; Wasim, M.; Rehmani, S.F.; Basharat, A.; Bibi, T. and Afonso, C.L. (2017): Repeated isolation of virulent Newcastle disease viruses in poultry and captive non-poultry avian species in Pakistan from 2011 to 2016. *Preventive veterinary medicine*, *142*, 1-6.

Wang, Y.; Duan, Z.; Hu, S.; Kai, Y.; Wang, X.; Song, Q. and Liu, X. (2012): Lack of detection of host associated differences in Newcastle disease viruses of genotype VIId isolated from chickens and geese. *Virology journal*, *9*, 1-15.

Wise, M.G.; Suarez, D.L.; Seal, B.S.; Pedersen, J.C.; Senne, D.A.; King, D.J. and Spackman, E. (2004): Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. Journal of clinical microbiology, 42(1), 329-338.

تقييم بعض برامج التحصين ضد مرض النيوكاسل من خلال دراسة تجريبية في دجاج التسمين

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هدفت الدراسة الحالية إلى تقييم فعالية برامج التطعيم المختلفة في حماية دجاج التسمين المُختبر ضد سلالة فيروس نيوكاسل VV. طُبّقت خلال التجربة لقاحات متنوعة حية ومُعطّلة ضد فيروس نيوكاسل، بما في ذلك لقاح هتشنر و كولون ١٢٤ و اللاسوتا، بالإضافة إلى لقاح مُعطّل. قُسّمت كتاكيت التسمين إلى خمس مجموعات؛ خضعت المجموعات الثلاث الأولى لبرامج تطعيم مختلفة ضد فيروس نيوكاسل، بينما أُبقيت المجموعتان الأخريان (المجموعتان ٤ و٥) دون تطعيم لتكونا مجموعتي الضبط الموجب والسالب. تم اجراء العدوى في اليوم الثامن والعشرين من العمر عن طريق الإعطاء عن طريق الأنف لفيروس النيوكاسل جينوتايب ٧ . حُدّدت عيارات الأجسام المضادة في الأيام ١، ٧، ١٤، ٢١، ٢٨، و٣٥ من التجربة. أشارت نتائج عيارات التساقط لفيروس النيوكاسل إلى أن أقل عيار طرح لوحظ في G3 وG2 (المطعمة باللقاح الحي والمعطل) وتبع ذلك في الأيام ٣ و٥ و٧ بعد التحدي في اليوم الثامن والعشرين، مقارنةً بـ G1 (المطعمة باللقاح الحي فقط). كما لم يتم تسجيل أي وفيات (معدل حماية ١٠٠٪) في المجموعة (٣) المطعمة بكل من اللقاحات الحية والمزدوجة من لقاحات فيروس النيوكاسل الميتة مقارنة بمعدلات الوفيات المنخفضة المسجلة في المجموعة (٢) المطعمة باللقاحات الحية وجرعة واحدة من اللقاح المعطل وتلك المطعمة باللقاحات الحية فقط. أشارت النتائج المسجلة إلى أن برامج تطعيم النيوكاسل التي تستخدم كل من الجرعات الحية والمزدوجة من اللقاحات المعطلة كانت أكثر فعالية من تلك التي تعتمد على جرعة واحدة من اللقاح المعطل مع اللقاحات الحية، وكذلك أكثر فعالية من البرامج التي تتكون فقط من اللقاحات الحية.

الكلمات المفتاحية: برامج التطعيم ، اللقاحات الحية والمعطلة ، مستويات الأجسام المضادة ، تساقط الفيروس