Research Article

Experimental Efficacy Evaluation of Different Vaccination Programs for Epidemic Newcastle Disease Virus in Egypt Against Challenge with Velogenic Genotype VII 1.1 in Commercial Broiler Chickens

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Abstract | Different Newcastle disease virus (NDV) vaccines and programs are heavily applied in poultry farms, but there is no marked protection against NDV genotype VII. So, the need of improved vaccines and vaccination protocols to reduce clinical disease and mortality is necessary. The current study evaluated the efficacy of different NDV vaccines used in poultry flocks in Egypt: live attenuated NDV vaccines (genotype II) and live recombinant herpes virus of turkey (rHVT-ND-IBD) alone or in combination with inactivated NDV vaccines either of genotypes II or VII (commercially available or autogenously prepared). Different vaccination regimes are applied at various designated days to 260 commercial broiler chickens in 13 groups; 20 birds each. At 28 days-old, all groups were challenged with NDV genotype VII 1.1 strain *"NDV-CHICKEN-EGY-ALEX-NRC-2020"* to evaluate the protective immunity of applied protocols. Humoral immune response, clinical signs, post-mortem gross lesions, growth performance as well as mortalities are all recorded and evaluated. All vaccination protocols were able to induce antibody levels for NDV after vaccination with varying titers. Overall, the serological response at challenge day were significantly with higher titers in groups vaccinated with inactivated NDV genotype VII vaccines when compared with other groups. Regarding broiler performance parameters it was noticed that, there is no significant difference in body weight (BW) as well as feed conversion rate (FCR) among all chickens in different vaccinated groups. From these findings, we concluded that vaccination programs include ND vaccines genotypically- matched to challenge virus in combination with live attenuated NDV vaccines provided a significant protection against mortality and clinical disease when compared with conventional vaccination regimes.

Keywords | Newcastle disease virus; Vaccination protocols; Growth Performance; Protective immunity; Genotype-matched vaccines.

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INTRODUCTION

NDV belongs to family Paramyxoviridae, subfamily Avulavirinae in genus Orthoavulavirus and also known as avian paramyxovirus 1 APMV-1) (Kuhn et al., 2013). NDV is an enveloped virus with a negative sense, single-stranded, non-segmented RNA genome encoding 6 structural proteins: nucleoprotein (NP) - phosphoprotein (P) - matrix protein (M) - fusion protein (F) – haemagglutinin neuraminidase (HN) - large polymerase protein (L). The F protein is one of the principal protective antigenic targets, mediates virus entry, cell-to-cell dissemination, and is also a major determinant for virulence (OIE, 2014). NDV was firstly diagnosed in Egypt as early as 1948 by Daubney and Mansy (1948). Presently and according to Rohaim et al. (2016) and Sabra et al. (2017), the common genotypes circulating in Egypt are NDV genotypes II, VI and VII, in which genotype II viruses are mainly of vaccine type and virulent genotype VI was mainly isolated from infected pigeons and clustered as pigeon paramyxovirus. While Radwan et al. (2013) who firstly isolated NDV sub-genotype VII in Egypt in 2012 and since that time was considered the predominant strain that has led to several outbreaks in poultry with high mortality rates in most of chicken farms in Egypt in spite of intensive vaccination strategies with both live and killed NDV genotype II vaccines (Orabi et al., 2017; Saad et al., 2017; Amer et al., 2019).

Inactivated, live attenuated and recombinant vaccines to control NDV are available in many countries, in which inactivated NDV vaccines can protect birds against clinical signs and to some extent induce a strong and long-lasting humoral response, which can be transferred to the offspring (Dimitrov et al., 2017). However, the inactivated vaccines are costly because they require an individual administration through subcutaneous or intramuscular injection (Al-Garib et al., 2003).

Live attenuated vaccines have the potential advantage inducing a strong humoral immune response with an early immunity onset of both cellular and mucosal immunity components and are often applied by using an aerosol spray, which reduces vaccine administration costs. Both types of vaccines can protect against clinical signs, and they also reduce the replication of the challenge virus in the host resulting in less viral shed (Martinez et al., 2018). Live attenuated vaccines however are negatively affected by maternal antibodies and can induce economically important vaccine reaction because of their replication in the respiratory tract (Westbury et al., 1984). Through the years, genotype II-based live attenuated vaccines as: La Sota, B1 have been used to reduce the risk of NDV in the global poultry industry (Miller, 2013). Moreover, these vaccines can protect against clinical disease but unable to control the mortalities and shedding of virulent isolates especially those belonging to genotype VII (Hu et al., 2011; Amer et al., 2019).

For the past 20 years, the use of recombinant vectored vaccines has been incriminated in the poultry industry largely because they can be used as in ovo or subcutaneous route in one-day-old chicks at the hatchery, and unlike live attenuated vaccines, they have no respiratory post-vaccination reaction (Rauw et al., 2010). The fowl pox virus (FPV) expressing the F or HN genes of NDV and the recombinant herpes virus of turkeys HVT expressing the F protein of NDV (rHVT ND) were found to protect chickens from challenge with virulent NDV and have been approved in many countries. Furthermore, rHVT ND vaccines have been shown to provide complete clinical protection three to four weeks post-vaccination with different virulent challenges (Palya et al., 2014).

Although, application of intensive vaccination regimens has raised, ND outbreaks still occurred periodically all over the world with extensive economic losses, mainly as high mortalities and decreasing of egg production rates; even in well-vaccinated flocks, have elevated questions about the phylogenetic variation of NDV genotypes and the protective efficacy of conventional vaccines (Cho et al., 2008). So, recent studies focused on the role of genotype-matched vaccines in the control of NDV outbreaks which found provide better protection and reduce virus shedding from infected birds against velogenic genotype VII NDV challenge (Hu et al., 2011; Yang et al., 2016; Amer et al., 2019).

On the base of dietary supplementation effect on growth performance, clinical signs and mortalities against viral challenge, Awadin et al. (2020) recorded that, dietary omega-3 supplementation for 4 weeks can improve growth performance and alleviate the deleterious immunological and pathological effects of NDV and avian influenza virus (AIV) H9N2 infection in quails. Furthermore, the oral administration of the mixed herbal extract for 5 weeks can stimulate the immune response to infectious bursal disease virus (IBDV) vaccination and relieves the pathogenicty of an AIV H9N2 and IBDV co-infection in chickens (Eladl et al., 2020).

The current study aimed to evaluate the efficacy of different vaccination protocols using commercial vaccines i.e. a live attenuated vaccine and a recombinant HVT expressing the fusion protein of NDV as well as inactivated NDV vaccines of both genotypes II and VII. The chickens vaccinated with these different vaccination protocols and were challenged with the ND virus genotype VII1.1 isolated in 2020 outbreak at 28 days-old and were evaluated for protection from clinical diseases, development of humoral

immune response, performance parameters and mortality rate.

MATERIALS AND METHODS

Ethics statement

This study was approved for experiment under the ethics of Medical Research Ethics Committee *(MREC)* of the National Research Centre, Egypt with approval number: (27210112021).

Chickens

Two hundred and sixty day-old commercial broiler chicks (Cobb 500®) were provided by certified local hatchery, divided into 13 separate experimental groups of 20 birds each. Each group was kept separately in isolated units with strict biosecurity level. Conventional animal food standards and welfare regulations were taken into account.

Chicken Ration

The chickens were fed on commercial rations according to the NRC (1994), and given pelleted starter (CP not less than 23%) and growing (CP not less than 21%) rations. The starter ration was used for the first 2 weeks and growing ration was used to the end of the experiment. Drinking water and rations were given to chickens *ad-libitum*.

ND challenge virus

The challenge virus was characterized by sequencing as velogenic NDV (vNDV) genotype VII1.1 designated as *"NDV-CHICKEN-EGY-ALEX-NRC-2020"* with an accession number of *(MW580389)* on GenBank. The virus was propagated via allantoic cavity inoculation of 9-day-old specific pathogen-free embryonated chicken eggs. The dose of challenge virus, equal to 6 Log 10 embryos infective dose (EID_{50}) per 0.5 ml and was administered intramuscularly to chickens (OIE, 2021).

Commercial Vaccines

1. *INNOVAX-ND-IBD*® frozen cell associated live virus vaccine contains recombinant turkey herpes virus serotype 3 with Newcastle disease virus F gene and Infectious bursal disease virus VP2 gene.
2. *PAMP* ® (H9N2+NDV genotype II, La Sota strain) inactivated oil emulsion bivalent vaccine, the dose of vaccine equal 8.2-Log-10 EID_{50} given 0.3 ml / bird.
3. *Vaxsafe*® live freeze dried NDV vaccine genotype II, V4 strain, the dose of vaccine equal 6log-10 EID_{50}/ bird.
4. Live attenuated NDV genotype II vaccine, La Sota strain with dose equal 6-log-10 EID_{50} / bird.
5. Live attenuated NDV genotype II vaccine, Hitchner strain with dose equal 6-log-10 EID_{50} / bird.
6. Inactivated oil emulsion NDV genotype VII1.1 vaccine *(Vaccine Valley®)* with vaccinal dose equal $10_{8.2}$ EID_{50} given 0.5 ml / bird. All vaccines were supplied by local agencies.

Autogenous vaccine preparation

Newcastle disease virus genotype VII 1.1 inactivated oil emulsion vaccine was autogenously prepared from purified and virulent *"NDV-CHICKEN-EGY-ALEX-NRC-2020"* strain with a concentration titer of 8.2 log10 EID_{50} as previously described in OIE (2021), the vaccine was prepared as an inactivated whole virus velogenic genotype VII 1.1 vaccine, where the viral antigen was propagated in SPF embryonated chicken eggs (ECE), then was harvested from the allantoic fluid and completely inactivated with ultra-purified formaldehyde 37% and further three passages in 10-day-old embryonated SPF chicken eggs were performed to the formalin-treated antigen to confirm the complete inactivation of the virus. All chicken embryos which injected with formalin-treated viruses survived after 120 hour, and no HA titer was detected. Finally, inactivated antigen was mixed with Montanide ISA-71 oil (Seppic Corp., Garenne-Colombes, France) adjuvant at a final concentration of 30/70 (v/v) following the manufacturers' instructions. The vaccine is prepared to be an experimental autogenous NDV genotype VII1.1 inactivated type. The safety and sterility quality control procedures are fully taken into consideration (OIE, 2021).

Haemagglutination inhibition (HI) assay

Sera were obtained from all birds pre-challenge at designated days and tested by HI assay. The HI assay was carried out using (LaSota strain) according to standard procedures with 4 Haemagglutinating units' virus/ antigen in 50 µl and HI titer ≤ 2 Log- $_2$ considered negative (OIE, 2021).

Broiler growth performance Parameters

Feed consumption and feed conversion rate were determined using the following Formula: Feed consumption (FC) g/bird = Feed intake in a replication / No. of live birds in a replication. Feed conversion ratio (FCR) = Feed intake (g) / Live weight (g). Growth performance Parameters were recorded for each chicken group from first to fourth week of age according to NRC (1994).

Experimental design for vaccine efficacy study

Two hundred and sixty day-old commercial broiler chicks were divided into 13 groups of twenty birds (G1 to G13) for vaccination with different regimes of NDV with live attenuated vaccines of genotype II or live recombinant NDV vaccines or inactivated genotype II NDV vaccines and Inactivated NDV genotype VII vaccines either commercially available or autogenously prepared by research team. G13 was provided as non-vaccinated challenged control. At 28 days old all groups were challenged with *"NDV-CHICKEN-EGY-ALEX-NRC-2020"* NDV belongs to velogenic genotype VII 1.1. (Table 1).

Table 1: Experimental design for evaluation of different NDV vaccination programs efficacy against challenge with velogenic NDV genotype VII.1.1at 28 days of age in commercial broiler chickens.

Group no.	Vaccination program		Assessment of protection
	Type	Age / days	
1	Live recombinant HVT ND [1] Inactivated ND H9 [2]	1 1	Seroconversion. Clinical signs. Post-mortem gross lesions. Mortality %. Broiler performance parameters
2	Live recombinant HVT ND Inactivated ND H9	1 5	
3	Live recombinant HVT ND	1	
4	Inactivated ND H9	1	
5	Inactivated ND H9	5	
6	Live NDV GII (V4 strain) [3]	14	
7	Live NDV GII (LaSota strain) [4]	14	
8	Live NDV GII (Hitchner strain) [5] Inactivated autogenousND (GVII strain) [6] Live NDV GII (LaSota strain)	5 7 14	
9	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) [7] Live NDV GII (LaSota strain)	5 7 14	
10	Live NDV GII (Hitchner strain) Inactivated autogenousND (GVII strain) Live NDV GII (LaSota strain)	5 10 17	
11	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) [7] Live NDV GII (LaSota strain)	5 10 17	
12	Live NDV GII (Hitchner strain) Live NDV GII (LaSota strain)	5 10 &17	
13	None vaccinated control	None	

[1] INNOVAX-ND-IBD® frozen cell associated live virus vaccine contains recombinant herpes virus serotype 3 turkey with the f Newcastle disease virus F gene and Infectious bursal disease virus VP2 gene which given subcutaneously.
[2] PAMP ® (H9+ND genotype II) inactivated oil emulsion bivalent vaccine. The dose of vaccine equal 8.2-Log-$_{10}$ EID_{50} given 0.3 ml / bird by subcutaneous route (S/C).
[3] Live freeze dried NDV vaccine genotype II V4 strain (Vaxsafe®), the dose equal 6log-10 EID50/ bird by oculonasal route.
[4] Live attenuated NDV genotype II vaccine La Sota strain. The dose equal 6-log-$_{10}$ EID_{50} / bird given by oculonasal route.
[5] Live attenuated NDV genotype II vaccine Hitchner strain. The dose of vaccine equal 6-log-$_{10}$ EID_{50} / bird given by oculonasal route.
[6]Autogenously prepared inactivated oil emulsion NDV genotype VII vaccine. The dose of vaccine equal 8.2-Log-$_{10}$ EID_{50} given 0.5 ml / bird by subcutaneous route (S/C).
[7] Inactivated oil emulsion NDV genotype VII vaccine (Vaccine Valley®). The dose of vaccine equal 8.2-Log-$_{10}$ EID_{50} given 0.5 ml / bird by subcutaneous route (S/C).
[8] velogenic Newcastle disease virus (genotype VII.1.1) used for challenge. The dose of challenged virus equal 6-Log-$_{10}$ EID_{50} given 0.5 ml / bird by intramuscular route (I/M).
Groups (n= 20 chicks each)

Table 2: Results of clinical signs with score in vaccinated and control groups after Velogenic Newcastle disease virus (Genotype VII.1.1) challenge at 28- days of age in broiler chickens (n= 20 chicken /group)

Group no.	Marked clinical signs				
	Greenish diarrhea	Nervous signs	Respiratory signs	Depression	Feed intake
1	Moderate	Moderate	Severe	Severe depression, dullness and ruffled feather	Marked decrease
2	Moderate	Moderate	Moderate	Moderate With dullness	Decrease
3	Moderate	Mild	Moderate	Depression and dullness	Highly decrease

4	Severe	Moderate	Severe	Severe depression, dullness and ruffled feather	Marked decreased
5	Moderate	Moderate	Moderate	Moderate	decrease
6	Moderate	Moderate	Moderate	Slight depression	Slight decrease
7	Severe	Severe	Moderate	Severe depression and ruffled feather	Marked decrease
8	Mild	None	None	Slight depression	Slight decrease
9	Mild	Mild	None	Slight depression	Slight decrease
10	Mild	None	None	Slight depression	Slight decrease
11	Mild	Mild	None	Slight depression	Slight decrease
12	Moderate	Moderate	Moderate	Moderate depression	Moderate decrease
13	Severe	Severe	Severe	Severe Depression and dullness With 100% mortality	Sever decrease Then off food

Table 3: Results of mortalities after vaccination with live inactivated oil emulsion NDV vaccines genotype (VII or II) and challenged with VNDV Genotype VII1.1) at 28- days of age in broiler chickens:

Group no.	Vaccination regime		Mortalities Post-challenge	
	Type	Age / days	No.	Percent
1	Live recombinant HVT ND Inactivated ND H9	1 1	15	75
2	Live recombinant HVT ND Inactivated ND H9	1 5	16	80
3	Live recombinant HVT ND	1	14	70
4	Inactivated ND H9	1	17	85
5	Inactivated ND H9	5	15	75
6	Live NDV GII (V4 strain)	14	16	80
7	Live NDV GII (LaSota strain)	14	15	75
8	Live NDV GII (Hitchner strain) Inactivated autogenous ND (GVII strain) Live NDV GII (LaSota strain)	5 7 14	2	*10
9	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) [7] Live NDV GII (LaSota strain)	5 7 14	4	*20
10	Live NDV GII (Hitchner strain) Inactivated autogenous ND (GVII strain) Live NDV GII (LaSota strain)	5 10 17	3	*15
11	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) Live NDV GII (LaSota strain)	5 10 17	4	*20
12	Live NDV GII (Hitchner strain) Live NDV GII (LaSota strain)	5 10 &17	13	65
13	Non vaccinated control	None	20	100

* Denotes significant difference from other groups post-challenge ($P < 0.05$).
HI titre ≤ 2 Log-$_2$ considered negative (OIE, 2012).

Statistical analysis

One way ANOVA with Tukey's post hoc test analyzed by SPSS 21 software is used to analyze the data and determine the significance of differences between individual treatments and corresponding controls. A probability (p) value ≤ 0.05 was considered statically significant.

Table 4: Results of serological response as measured by HI test after vaccination of chicken groups (n= 20 chickens/ group) with different live and inactivated NDV vaccines genotype (VII or II) and challenged at 28 days old

Group no.	Vaccination regime		HI titre means SD Log-2 at age / days (N = 10)			
	Type	Age / days	7	14	21	28
1	Live recombinant HVT ND Inactivated ND H9	1 1	6.00±1.41	3.8±0.83	3.8±0.83	5.2±0.83
2	Live recombinant HVT ND Inactivated ND H9	1 5	6.4±0.89	3.8 ±0.83	4.4 ±1.14	5.4 ±0.83
3	Live recombinant HVT ND	1	6.4±0.55	3.4±0.54	3.6±0.83	3.2±0.54
4	Inactivated ND H9	1	5.6 ±0.54	4.00 ±1.00	4.00 ±0.70	4.6 ± 0.89
5	Inactivated ND H9	5	6.4 ±0.54	3.8±0.83	4.2± 0.83	4.5± 0.83
6	Live NDV GII (V4 strain)	14	6.4 ±0.54	3.9 ± 0.83	5.6± 0.54	4.8. ± 0.70
7	Live NDV GII (LaSota strain)	14	6.2 ± 0.83	3.8±0.83	4.6±0.54	5.2± 0.89
8	Live NDV GII (Hitchner strain) Inactivated autogenous ND (GVII strain) Live NDV GII (LaSota strain)	5 7 14	5.8 ±1.30	5.4±0.54	5.8 ±0.70	*7.4±0.89
9	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) Live NDV GII (LaSota strain)	5 7 14	5.6 ±0.54	5.2±0.83	5.6 ±0.54	*6.8±1.48
10	Live NDV GII (Hitchner strain) Inactivated autogenous ND (GVII strain) Live NDV GII (LaSota strain)	5 10 17	5.00±0.70	5.00 ±0.54	5.8±0.54	*7.2±0.70
11	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) Live NDV GII (LaSota strain)	5 10 17	5.4 ±0.54	4.6 ±0.70	5.2±1.30	*6.6±0.70
12	Live NDV GII (Hitchner strain) Live NDV GII (LaSota strain)	5 10 &17	5.6 ±1.00	4.1±0.54	4.3±0.83	5.00±1.22
13	Non vaccinated control	None	5.00±1.00	3.4±0.54	2.00±0.70	0.6±0.54

* Denotes significant difference from other groups at 28 days old ($P < 0.05$).
HI titre ≤ 2 Log- $_2$ considered negative (OIE, 2012).
N number of tested samples

Table 5: Results of performance parameter after vaccination with live and inactivated NDV vaccines genotype (VII or II) and challenged with Velogenic Newcastle disease virus (Genotype VII1.1) at 28- days of age in broiler chickens.

Group no	Vaccination regime	Performance parameters							
		Day 7		Day 14		Day 21		Day28	
		BW M±SD	FCR	BW M±SD	FCR	BW M±SD	FCR	BW M±SD	FCR
1	Live recombinant HVT ND Inactivated ND H9	175±15	49.5	483.5±47.9	16.5	957±113	14.6	1572.4±103.6	15.9
2	Live recombinant HVT ND Inactivated ND H9	167.6±12.4	33.7	481±89	16.6	1027±64.4	13.6	1604±155.7	15.8
3	Live recombinant HVT ND	177.3±14.6	29.8	510.5±23	14.1	1014±62.8	12.5	1606.3±148.6	15.5
4	Inactivated ND H9	162.6±16.2	32.8	449.6±50.9	19.3	976.3±62.8	14.3	1558.7±113.7	16.04
5	Inactivated ND H9	158.8±9.7	29.5	435±26.5	19.5	978.4±75	14.3	1586.7±136.9	16.1
6	Live NDV GII (V4 strain)	158±11.3	22.7	433±25.1	20.5	978.7±69.9	14.3	1586.5±145.4	16.4

7	Live NDV GII (LaSota strain)	160.6±14.3	27.3	423.2±40.5	21	1039±98.1	13.4	1561±141.3	16.01
8	Live NDV GII (Hitchner strain) Inactivated autogenously ND (GVII strain) Live NDV GII (LaSota strain)	150.5±13.3	30.5	436±39.2	13.7	1009±112.1	13.8	1624.7±183.3	15.7
9	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) Live NDV GII (LaSota strain)	150.4±13.5	30.5	452.5±38.4	13.9	1073±87.1	13	1642.6±160.1	15.5
10	Live NDV GII (Hitchner strain) Inactivated autogenously ND (GVII strain) Live NDV GII (LaSota strain)	148.5±15.3	26.9	451.5±36.5	15.5	1070±85.8	12.1	1592±124.4	14.4
11	Live NDV GII (Hitchner strain) Inactivated commercial ND (GVII strain) Live NDV GII (LaSota strain)	150.3±11.7	22.6	466±24.1	16	1057±128.4	13.24	1627±206.7	14.1
12	Live NDV GII (Hitchner strain) Live NDV GII (LaSota strain)	152.6±10.6	19.6	450±64.8	16.6	1081±99.5	12.9	1631±165.2	15.3
13	Non vaccinated control	152.6±19.2	28.4	434±38.7	20.1	1076±123.8	13	1607±163.3	14.9

GP no: group number
BW: body weight
FCR: feed conversion ratio.

Statistical analysis

One way ANOVA with Tukey's post hoc test analyzed by SPSS 21 software is used to analyze the data and determine the significance of differences between individual treatments and corresponding controls. A probability (p) value ≤ 0.05 was considered statically significant.

RESULTS

Efficacy study of experimental challenge vaccination in commercial broiler chickens

All birds in non-vaccinated challenged control group G13 died all by day 4 post-challenge (pch) developing severe depression, respiratory and nervous signs with marked greenish diarrhea then off-food hours before death. While G1, G2, G3, G4 and G5 vaccinated with either live recombinant NDV vaccine or inactivated NDV vaccines or both recorded 75%, 80%, 70%, 85% and 75% mortalities, respectively pch showing varying degrees of moderate or severe respiratory and clinical signs with greenish diarrhea associated with decreased food intake. In addition to, G6 and G7 receiving live attenuated NDV GII vaccines (V4 and LaSota strains) are of 80% and 75% deaths, respectively pch displaying moderate respiratory and nervous signs with greenish diarrhea and slight to marked decrease in food intake. Furthermore, vaccinated groups G8, G9, G10 and G11with live attenuated NDV vaccines GII (Hitchner and LaSota strains) and inactivated NDV vaccines GVII at different designated days recorded significantly lower mortalities and much less clinical signs in compared with other groups which found 10%, 20%, 15% and 20% mortalities, respectively pch. Finally, G12 vaccinated only with live attenuated NDV GII vaccines of both Hitchner and LaSota strains showed 65% deaths and displayed moderate signs of either respiratory or nervous accomplished with greenish diarrhea and decreased food intake (Table 2 & 3). At necropsy, petechial hemorrhages were found on tips of periventricular glands, cecal tonsils ulceration with splenomegaly and severe hemorrhagic tracheitis in non-vaccinated infected controls. While, similar but milder gross

lesions were observed in vaccinated challenged groups G1, G2, G3, G4, G5, G6 and G7. No obviously gross lesions recorded in vaccinated challenged groups G8, G9, G10 and G11.

Serological response measured by HI assay

Mean HI titers obtained for commercial broilers post-vaccination in all vaccinated and non-vaccinated controls are shown in (Table 4 & Figure 1) at 7, 14, 21 and 28 days of age. All vaccinated groups G1 to G 12 revealed positive HI titers for NDV, which mostly increased throughout the vaccination course with significant higher titers from control group C at all designated tested days. In which, G1, G2, G3, G4 and G5 mean HI titers at 7 days old were ranged from 6.00 to 6.4 and found to be 3.2 to 5.8 HI range at challenge day. While, G6 and G7 showed mean HI titer range of 6.2 to 6.4 and 4.8 to 5.2 at 7 and 28 days old, respectively. Otherwise, G8, G9, G10 and G11 recorded the highest significant mean HI titer at all tested days in compared with other groups especially at challenge day with 7.4, 6.8, 7.2 and 6.6 titers, respectively. Lastly, G12 group displayed reasonable HI mean titers ranged from 5.6 to 5.00 at 7 and 28 days old, respectively.

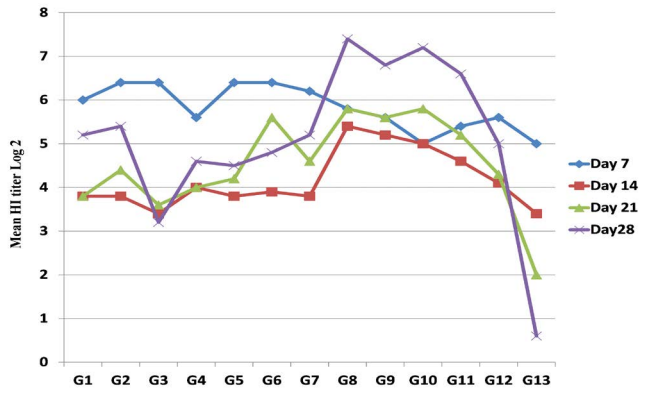

Figure 1: Serological response dynamics of vaccinated-challenged groups and non-vaccinated infected control groups. Mean HI- titers (log-2) for sera collected at days 7, 14, 21 and 28 of age.

Performance parameters

Average body weight (BW) and feed conversion rates (FCR) in all vaccinated and non-vaccinated controls are shown in (Table 5 & Figure 2) at 7, 14, 21 and 28 days of age. Average BW in groups 1,2,3,4 and 5 were ranged from 158.8 gram (gm) to 177.3 gm in 1st week and 1550.7 gm. to 1606.3 gm in 4th week, respectively.

While, average BW in groups 6 and 7 were 158 gm., 160.6 gm in 1st week and 1486.5 gm, 1421 gm in 4th week, respectively. Furthermore, average BW in groups 8,9,10 and 11 were ranged from 148.5 gm to 150.5 gm in 1st week and 1592 gm to 1682.6 gm in 4th week, respectively. Although, the groups 8,9,10 and 11 recorded the highest increase in BW, it is not significant increase.

Finally, group 12 averages BW were 152.6 gm, 1631 gm in 1st and 4th week, respectively before challenge.

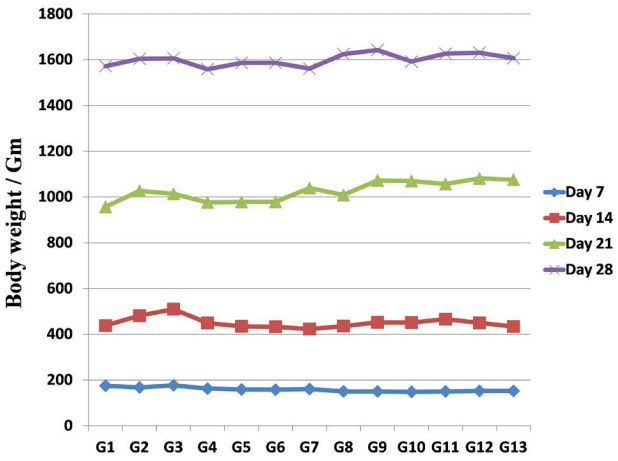

Figure 2: Average body weight gain of vaccinated-challenged groups and non-vaccinated infected control groups at days 7, 14, 21 and 28 of age

DISCUSSION

Velogenic NDV continues to be a problematic disease for the poultry industry in spite of the widespread application of different vaccines for years ago. Despite of all NDV isolates belongs to a single serotype, as antibodies against NDV isolates should provide protection against other NDV isolates, but any NDV strain vaccination couldn't provide the same protection against all NDV isolates (Shahar et al., 2018). The antigenic variations not still providing cross-protection between NDV genotypes have been proposed the reason why vaccines have not been more protective for the poultry flocks, So the better clinical protection is afforded when using antigenicaly-matched NDV vaccines to the challenge virus (Miller et al., 2009; Cornax et al., 2012; Amer et al., 2019). Subsequently in the current study, protection levels induced by live attenuated vaccines, live recombinant rHVT ND vaccine and inactivated vaccines of both genotypes II and VII were all compared in a broad vaccination regimen following challenge with NDV genotype VII1.1 isolate homologous to some vaccination protocols and heterologous to others in antigenic structure. Chickens vaccinated against NDV with live vaccines develop immunity subsequently early and neutralizing antibodies can be determined 6-10 days post- vaccination (Al-Garib et al., 2003; Kapczynski et al., 2013). While, protection induced by inactivated vaccines is expected to reach peak near 3 weeks after the primary vaccination shoot and then start to decrease later on (Vrdoljak et al., 2017). Furthermore, live recombinant HVT vaccines expressing

the fusion protein of NDV usually provide sufficient protection in chickens against morbidity and mortality after NDV challenge (Palya et al., 2012; 2014; Kapczynski et al., 2015). In addition, it has proved that obtaining the solid polyvalent protection by combination of these recombinant vaccines is difficult due to the interference between different HVT construct vaccines combine (Dunn et al., 2019).

Although chickens have not been be infected in field through injection, it is the recommended route for challenge, as the oculonasal route of infection does not permit for exacting delivery of infective virus to each bird (Alexander and Senne, 2008). In our experiment, live attenuated NDV vaccines was applied primarily at age of 5 days to avoid its probable neutralization by maternal antibodies which are decreased by half every 4-5 days. While, inactivated vaccines were inoculated earlier and later on as less affected by maternal antibodies and producing higher neutralization titers as previously discussed by (Umino et al., 1987; Miller et al., 2009).

Clinical signs related to infection with vNDV in the challenged birds include usual symptoms such as; depression, greenish diarrhea, tremors and respiratory manifestations which in most cases led to mortality, however a number of vaccinated birds showed mild to moderate signs and were found fully recovered by the end of the observation period, especially in groups 8, 9, 10 and 11 (genotype VII) that were apparently more protected against clinical signs compared to other groups as previously mentioned by (Cho et al., 2008; Vrdoljak et al., 2018; Amer et al., 2019). On the base of vaccination against clinical disease, Cornax et al. (2012); Dortmans et al. (2012); Kapczynski et al. (2013) mentioned that, vaccination of chickens with live and/or inactivated genotype II strains, providing adequate protection against clinical disease against vNDV challenge. While, Hu et al. (2009) concluded that, no obvious clinical signs were found in birds vaccinated with either inactivated NDV genotype VII or genotype II vaccines after challenge with vNDV genotype VII. In addition, similar findings suggested that, both inactivated genotype VII and genotype II vaccines provided similar protection from clinical disease against challenge with NDV genotype VII (Yang et al., 2017). On the other hand, Cho et al. (2008); Hu et al. (2011); Roohani et al. (2015) reported that, vaccination with genotype VII NDV inactivated vaccine provided better protection against morbidity and clinical disease, in which no clinical signs of ND were observed for chickens vaccinated with inactivated genotype VII NDV vaccine as recorded in our study with significant protection against mortality and clinical disease in groups vaccinated with inactivated genotype VII vaccines.

As well as clinical signs, post-mortem gross lesions are essential in assessment of protection against vNDV genotype VII challenge, which commonly seen as; hemorrhages in the proventricular glands, spleen, trachea, cecal tonsils and bursa, In addition to the spleen found to be enlarged, mottled and necrotic (Wakamatsu et al., 2006; Susta et al., 2011; Miller and Koch, 2013). Similar findings to these studies were detected in the present study which recorded as; characteristic enlarged and mottled spleen and hemorrhagic spots on tips of proventriculus in most of vaccinated groups with little or no lesions in genotype VII vaccinated groups compared with non-vaccinated challenged (G13) showing severe gross lesions including; severe congestion in the trachea, liver, mottled and enlarged spleen, hemorrhagic spots on tips of the periventricular glands and enteritis with greenish intestinal contents. Here in, concluding that NDV vaccines based on combination between genotype II and VII genotypes could provide an adequate protection from serious post-mortem changes against VNDV challenge as discussed also by (Yang et al., 2017).

One of the most direct tools to estimate the protection is HI antibody titer induced by ND vaccines, as it corresponds with level of protection, in which HI titers of 6 Log-2 or higher are what typically thought of being protective (Raghul et al., 2006). The current investigation confirmed this finding and further revealed that G8, G9, G10 and G11 recorded the highest significant mean HI titer at all tested days when compared with other groups especially at challenge day with 7.4, 6.8, 7.2 and 6.6 titers, respectively. This finding emphasized that the NDV genotype VII based vaccine could be effective against vNDV genotype VII infection when compared to genotype II and live recombinant rHVT vaccines especially when HI titers are below the protective levels as in agreement with (Cho et al., 2008; Hu et al., 2011; Roohani et al., 2015). On the contrast, no obvious differences in HI titers, regarding the level of protection against VNDV genotype VIId challenge between inactivated NDV genotype VII and genotype II vaccines, as previously detected by (Hu et al., 2009; Yang et al., 2017).

Chickens growth performance which can be affected by vaccination as feed conversion and body weight gain are very important especially in broilers. Moreover, our investigation showed no significant effect on the production parameters when healthy chickens were vaccinated with different live and /or inactivated NDV vaccines as in agreement with (Okwor et al., 2013). Furthermore, Martinez et al. (2018) concluded that, there is no significant differences in FCR were noted between vaccinated and non-vaccinated chickens. In the same side, another study carried out by Costa-Hurtado et al. (2015) reported no differences in BW between control and vaccinated groups. While on the oth-

er side, Albarrak et al. (2021) reported that final BW and the BWG in vaccinated chickens with La Sota and VG/GA strain were significantly increased compared to unvaccinated control group. In addition, there were differences in BW of infected vaccinated birds with NDV compared to BW of birds unvaccinated and infected with NDV that unvaccinated birds was accompanied by a significant decrease in BW than infected vaccinated birds (Ellakany et al., 2018). While, Wang et al. (2015) concluded that, ND vaccine (LaSota) in earlier stage of growth decreased body weight and feed efficacy, later on with different 6 doses of LaSota treatment down-regulated the feed gain ratio by 6.36 % during the whole growing period.

CONCLUSION

The results of vaccination efficacy study indicated that; vaccination programs based on NDV genotype VII matched to the challenge virus provided significant serological response and protection against mortality when compared to classic vaccines (genotype II, recombinant rHVT) which are phylogenitically-divergent from challenge virus. Moreover, further studies are needed to introduce more intensive vaccination regimens in broiler vaccination programs to achieve better protection against currently epidemic vNDV infection.

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AUTHOR'S CONTRIBUTION

All authors equally participated in design, experimental procedure, writing, revised, and reviewing the manuscript.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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