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Avian Diseases

Efficacy of Commercial (HVT-ND) Vector Vaccine Against Newcastle Disease Virus Genotype VII.1.1 Challenge in Commercial Broiler Chickens

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ABSTRACT

Genotype VII Newcastle disease viruses (NDV) are still circulating in Egypt despite extensive vaccination programs. In our study, we evaluate the protective efficacy of commercial (HVT- ND) vector vaccine against velogenicNDV GenotypeVII.1.1challengein broiler chickens. Five vaccination strategies were established; G1 vaccinated by HVT-ND vaccine, G2 vaccinated by HVT-ND and 2 live ND vaccines, G3 vaccinated by HVT-ND, inactivated ND and 2 live ND vaccines, G4 vaccinated by 2 live ND vaccines, G5 vaccinated by inactivated ND and 2 live ND vaccines and G6 Non-vaccinated challenged. All birds were challenged atthe age of 28 days-old. The protection evaluation based on mortality, clinical symptoms, gross pathological lesions, immune response and the mean lesion scores (MLS) of histopathological lesions in brain and trachea at 14 days post challenge.Our findings showed that mortality rates were 26.6 %, 16.6%, 30% and 3.3%, respectively, in G1, G2, G4 and G5,while the best protection was in G3 that did not show any mortality versus 93.3 % in G6. Moreover the MLS on 7dpc were 1.75, 1.6, 1.18 , 1.65 and 1.23, in G1, G2, G3, G4 and G5,respectively,while it reached to 2.5 in G6. Results of virus shedding in all vaccinated groups significantly reduced while (G3) also show the more reduction of virus shedding. It could be concluded that full clinical protection against challenge with genotype VII.1.1. NDV can be achieved by co-administrating ofHVT-ND, inactivated ND and live ND vaccines as a vaccination program in endemic regions.

Keywords: HVT-ND vaccine, Genotype VII.1.1, Inactivated vaccine and Newcastle disease virus.

INTRODUCTION

Newcastle disease (ND) is a highly contagious and often severe of the more serious diseases threatening worldwide raising of poultry production for what it causes a disastrous losses in producers investments resulting from extensive weight losses, poor feed conversion and severe mortality rates in broiler chickens and severe decline in egg production quantity & quality in commercial layer chicken farms (Diel et al., 2012). Genetically, NDV related to negative sense RNA viruses with single stranded, non-segmented coding for at least six structural proteins (Miller et al., 2013 and Toyoda et al., 1987). It was classified into two main classes, class I and class II at which viruses of class II were divided into 18 genotypes expressed as Latin numbers, while Class I viruses grouped as a one genotype that only infects mammalian and reptilian species (Millerand Koch, 2013). Recently, a uniform phylogenetic categorization system was updated, and the nomenclature for NDV was altered at which three new classes II genotypes have been added associated with reduction of

sub-genotypesnumber to obtain XXI - NDV genotypes within class II. Egypt became endemic with velogenic NDV strains and according to more recent classification, the majority of recent Egyptian NDV isolates belong to genotype VII 1.1 due to mutations at various F gene sites, such as in the N-glycosylation sites, binding epitope sites, and cysteine residues, which primarily affect virus pathogenicity and may conflict with the protection provided by the earliest classical conventional vaccines (Radwan et al., 2013; Sultan et al., 2019 and Moharam et al., 2019). ND continues to be one of the most significant avian diseasesand affects major poultry farms in numerous countries, despite the development of numerous vaccine types and immunization regimens for these viruses for control more than 60 years ago (Aini et al., 1990).Available vaccines which are mostly of genotype I & II could partially or completely protect chickens from mortalities and clinical signs but the extent of shedding of virus to the environment depends a lot on the characteristics of vaccine used, the quality of vaccination procedures and influenced also by the genotype of the challenge virus (Miller et al., 2007 and Palya et al., 2021). Different countries in different parts of world have employed more recent NDV vaccinations. These new vaccinations are based on LaSota NDV vaccines that have been reverse genetically modified and herpes virus-vectored to express velogenic F and/or HN genes(Li et al., 2016). Types of vaccines used to immunize against NDV include live, inactivated and the newest recombinant vaccines (Mansur-Ud-Din et al., 2007). Currently, many commercially available HVT-vectored vaccines are constructed and used efficiently (Esaki et al., 2013; Kapezynski et al., 2015 and Ingrao et al., 2017). They are now used to protect raised poultry flocks against a different numbers of veryimportant diseases such as Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), laryngotracheitis infection virus (ILTV), and avian influenza virus.

Within this study, we wanted to see how well the HVT-ND vaccination protected against a challenge with velogenic NDV genotype VII.1.1. (NDV Chicken/ USC/ Egypt/ ElGharbia/ 2015). Also to assess the need for co-administrating live ND vaccination with inactivated ND vaccine in addition to HVT-ND vaccine against challenge with velogenic NDV genotype VII.1.1. in broiler chickens

MATERIALS AND METHODS

Vaccines:

HVT-ND vaccine (Poulvac® Procerta™ HVT-ND expressing the F protein from a genotype II, clone-30 strain of Newcastle disease virus (NDV) obtained from Zoetis, Charles city, USA), Live V4 strain virus vaccine against Newcastle disease virus, vaccinal dose 10^6 EID_{50} obtained from Zoetis, Australia, Live LaSota strain virus vaccine, genotype II, against Newcastle disease virus (NDV), vaccinal dose contains 10^6 EID_{50}, obtained from Zoetis, Charles city, USA), Inactivated oil emulsion genotype II NDV vaccine against Newcastle disease virus, vaccinal dose contain ND Clone 30 ≥ 50 PD_{50} obtained from MSD, Spain.

Challenge Virus:

The challenge virus employed in this investigation was a previously reported and characterized as NDV GVII virus (NDV Chicken/ USC/ Egypt/ El Gharbia/ 2015), accession number (KM659400).

Experimental Design*:*

Three hundred commercial broiler chicks of one day old thathaving maternal NDV anti-bodies,the chicks weregiven from a local hatchery where it's breeder had been vaccinated with conventional inactivated NDV genotype II vaccines for five times (at the age of 10, 48, 69, 90 and 120 days of life) and seven live ND genotype II vaccines (at the age of 10, 38, 60, 85,105, 135, and 170of life). The chicks wererandomly classified into 6 groups (G1 to G6), each with 50 birds (Table 2). In this experimental investigation a routine immunization schedule for commercial broilers in Egypt was used where

G1 vaccinated only by HVT-ND inoculated in hatchery via subcutaneous route in day old chicks, G2 vaccinated with HVT-ND and 2 NDV live vaccines administrated via eye drop. While G3 vaccinated with inactivated oil emulsion genotype II NDV vaccine against Newcastle disease virus administrated by a dose of 0.5 ml through S/C, besides HVT-ND and 2live NDV vaccines, G4 vaccinated only with the 2 live NDV vaccines, G5 vaccinated with inactivated vaccines with 2live NDV vaccines and G6 Non-vaccinated. All chicks that are used in our experiment were kept in separate pens with a completely access to food and water on a daily basis in the samesuitable environmental circumstances of temperature and relative humidity at completely biosecure experimental facility. Challenge with VNDV GVII strain was given to birds in all six groups,10^6 EID_{50} per bird via the intramuscular route. Cloacal and tracheal swaps were collected from all vaccinated and non-vaccinated groups (20 tracheal and cloacal swab of birds /group). Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used before the challengeby using common NDV primer sets (Leary et al., 2013).The primers used shown in (Table1).For 10 dpc, clinical symptoms were observed in all of the experimental groups and recorded.Tracheal and cloacal swabs were obtained at 2, 4, and 7 dpc to measure viral shedding.

Serology:

Hemagglutination inhibition (HI) assay and an ELISA test using commercial ELISA kits ID. vet ID Screen NDVsupplied by ID.vet. Corporation, 310, rue louis Pasture-Grabls France. The two tests were used to measure the serum antibodies to Newcastle disease virus. Blood samples were taken from birds in the age of 7 and continued to be collected at weekly interval 14, 21, 28, 35, and 42 day of life. These samples were serum separated and kept at -20°C until analysis. Four hemagglutination units (4HAU) of the NDV LaSota antigen were used in our laboratory measures. HI titers were expressed in terms of log2, and samples showing HI titers that were equal to or over 4-log2 were considered to be positive (Al-Habeeb et al., 2013).

Histopathological Examination:

Brain and trachea tissue samples were taken, treated by 10% buffered formalin for around 20 days, processed for histology, and stained with hematoxylin and eosin (H&E) (Gibson et al., 2013). Lesions of infected tissues were rated according to their severity, with 1 denoting no lesions, 2 for mild lesions, 3 moderate lesions, 4 severe lesions, and 5 extremely severe lesions (Sultan et al., 2019). The mean of the five individual optical fields was obtained after five random optical fields were viewed and scored. The measurement of hisopathological modifications can be measured by the function sum of the mean lesion scores of the three inspected organs from three chickens/group at 4 and 7 dpc divided by the total number of analyzed organs from all chickens was used to construct the mean severity index (MSI), as previously stated by (Bancroft and Stevens. 1977).

Virus shedding:

At 2, 4, and 7 dpc, tracheal and cloacal swabs from 20 distinct birds/group were taken and placed in 400 µl of Dulbecco's modified Eagle medium with an antibiotic solution. Then, according to the manufacturer's recommendations, viral RNA was directly extracted using the TRIzol reagent (Gibco, Invitrogen, Carlsbad, CA, USA) and suspended in (DEPC) water. Using particular primer showing in (Table, 1) and probe sets from (QIAGEN, Valencia, CA, USA), standard RT-PCR was carried out. The QuantiTect RT-PCR Master Mix, 0.25 µl of the QuantiTect RT Mix, 0.25 µl of each primer (50 pmol concentration), 0.125 µl of each probe(30 pmol concentration), 3.625 µl of PCR-grade water, and 7 µl of RNA template were used in the RT-PCR test. A30-minute setup period at 50 °C was followed by a 15-minute primary denaturation step at 94 °C, 40 cycles of denaturation at 94 °C for 15 s, annealing at 54 °C for 30 s, and extension at 72 °C for 10 s. The reaction performed with an MX3005P real-time PCR apparatus (Stratagene, La Jolla, CA, USA). Real time reverse transcription (RRT-PCR) titers were converted into $\log_{10}EID_{50}$/ml, as

described before (Nolan, 2020). The usual system detection limit, previously stated as 0.5 EID_{50}/ml (Sultan et al., 2020). The Ct of unknown samples was plotted against the main standard curve to determine the amount of Newcastle disease virus present, which was then quantified in terms of log_{10} EID_{50}/ml equivalents.

Statistical Analysis:

When it was important to assess the means of significance differences between individual treatments in the immunized groups and the equivalent individuals in controls, data analysis may be done by one of using Student's t-test or ANOVA, then Duncan's new multiple range test is then applied (Steel and Torrie, 1960).

RESULTS

Clinical signs, Postmortem, Gross Lesion and Mortalities:

The birds in G3, showing13 % (4/30) mild respiratory disorders, and 10 % (3/30) showed a greenish characterized diarrhea, but without ocular disorders or nervous signs. Allgroup recovered within a period of short time, reporting 100% (30/30) protection within the period of examination without any mortality (Table3). In the same manner, G-5, showed 23.3% (7/30) with mild respiratory signs, and 20% (6/30) greenish diarrhea, while no eye lesion or nervous signs with 96.7% (1/30) clinical protection, other groups as G-1 showed variable degree of respiratory signs 60% (18/30), diarrhea 53% (16/30), eye lesion 13% (4/30) and 3%(1/30) nervous signs with mortality 26.6% (8/30), while, G2 showed 16.6% (5/30) mortality with 46% (14/30) respiratory signs, Greenish diarrhea 40%(12/30) with 3.3% (3/30) eye lesion without nervous signs (Table3). In G4, 67 % (20/30) of the birds suffering from respiratory disorders and nasal discharge. Additionally, 73% (22/30) of the birds exhibits a watery greenish diarrhea with some ocular lesions 16% (5/30), and recovered to indicate 67% (20/30) protection and showing30% (9/30) mortality rate. In G6,100% (30/30) of the affected chickens showed moderately or severe respiratory symptoms, while 83% (25/30) showed greenish watery diarrhea and16 % (5/30) showed different forms of nervous disorders and paralysis. Mostly all birds died (28/30) with high mortality rate reached 93.3%.

The proventriculus of the dead birds in groups G-1, G-2, and G-4 had minor petechial hemorrhages compared to G-6 that has severe petechial hemorrhages. As well as mild focal necrosis in the spleens of the birds from Groups G-1, G-2, and G-4 but very severe necrosis and moderate to severe hemorrhagic ulcer in the caecal tonsils have been seen in the birds from G6.Groups (G-3 and G-5) showed no or mild gross lesion in proventriculus, trachea, lung, spleen, and caecal tonsils.

Serum antibody response to vaccination:

The HI results at day of challenge showed significant increase in G3 and G5; 5.7 and 7.1 log_2, respectively when compare with other groups 3 , 4.6 and 5.4 log_2 in G1 , G2 and G4, respectively versus 1.8 in G6 (Non-vaccinated challenged).The result of ELISA test show nearly the same result afterthe challenge of NDV,theHI and ELISAtitersofall vaccinated groups exhibits an increase in the serological immune response at 35 and 42 day of age as shownin (Fig 1&2).

Histopathology:

Themeanlesionscores(MLS) of all examined organs were 2.8, 2.5,3 and 2 from the dead birds of G1, G2, G4 and G5, respectively.While the organs were very histologically altered with severelesion scores in control challenged group (G6) with the higher MLS = 4but, there is no dead birds in G3.

In addition the result at 7 dpc revealed the same similar lesions cores where (MLS) were 1.75, 1.56, 1.18, 1.65 and 1.28 for G1, G2, G3, G4 and G5, respectively, versus 2.5 in G6. ($p< 0.05$), as shownin (Fig. 5) and (Table 4).

Virus shedding:

The results are shown in (Fig.3&4). On the 2nd DPC, the birds in G1 demonstrated that the virus titers in the tracheal and cloacal swabs, was 3.2 and 3.85, respectively, while in G2 it was 3.66 and 2.62, respectively. The birds in G3 showed tracheal and cloacal viral titers of 2.7 and 2.04, while, in G4 were 3.62 and 3.8, respectively. The birds in G5 showed the tracheal and cloacal viral titers of 3.8 and 4.2 versus G6 showed tracheal and cloacal viral titers of 4.5 and 5.2, respectively. On 4th DPC , the birds in G2 and G3 displayed lowest tracheal and cloacal viral titers then G1, G5 and G4 when compared to G6 that had tracheal and cloacal viral titers of 5 and 6.8, respectively.On the 7thDPC, the birds in G3 did not show any shedding either from tracheal or cloacal and G5 birds only displayed cloacal shedding. Birds in G1, G2, and G4 showed that the tracheal and cloacalvirus titers were at 2.18 and 2.2, 2.11 and 2.10, &2.44 and 2.17, respectively, While G6 had tracheal and cloacal titers of 5.5 and 7, respectively.

Table (1). The oligonucleotide primers that used for the NDV F-gene amplification:

Gene	Primer used	PrimerSequences5^J-3^J	Reference
NDV F-gene	Forward	5^J-TCCGGAGGATACAAGGGTCT-3^J	(Learyet al., 2013).
	Reverse prob	5^J-AGCTGTTGCAACCCCAAG-3^J 5^J-FAM-AAGCGTTTCTGTCTCCTTCCTCCA-BHQ1-3^J	

Table (2): Experimental grouping, used vaccines and vaccination regimes as well as assessment of immune response in commercial broiler chickens:

GroupNo.	**BirdsNo.**	**NDVvaccinationregime**		**Challenge[1] Age/day**	**Assessment ofprotection**
		Vaccine genotype	**age/days**		
1	50	**rHVT[2]- ND**	1		**1.** Clinicalsigns **2.** PM.grosslesions **3.** Mortality% **4.** Seroconversion **5.** Detectionofvirals hedding **6.** Histopathology
2	35	**rHVT- ND** **[3]Live G-II** **[4]Live G-II**	1 1 14	32	
3	35	**rHVT- ND** **Live G-II** **Live G-II** **[5]Inact, G-II**	1 7 14 7		
4	35	**Live G-II** **Live G-II**	1 14		
5	50	**Live G-II** **Live G-II** **Inact, G-II**	1 14 7		
6	50	**-**			

[1]Challenge with VNDV genotype VII.1.1 (Chicken/USC/Egypt/2015), EID_{50} = 10^6 / 0.5 ml / bird via I/M route
[2]HVT-ND expressing the F protein of genotype II clone-30 strain of Newcastle disease virus (NDV).
[3]Live V4 NDV strain with vaccinal dose 10^6EID_{50} and administrated via eye drop.
[4]Live LaSota NDV strain vaccine, of genotype II vaccinal dose contains 10^6 EID_{50} administrated via eye drop.
[5]Inactivated oil-emulsion genotype II NDV vaccine, vaccinal dose contain ND Clone 30 ≥ 50 PD_{50} administrated 0.5 ml by S/C rout.
- no = Number.

Table (3). Mortality and clinical signs in vaccinated and control challenged groups on the age of 28 day with VNDV genotype VII.I.I:

Groups	**Lesion of Eye**	Respiratory signs	**Diarrhea**	**Nasal Discharge**	**Paresis**	**Nervous signs**	**Mortality. (%)**
G1	0/30[a]	18/30[b]	16/30[b]	8/30[a]	1/30[b]	1/30[b]	**26.6[a] %**
G2	3/30 [a]	14/30[b]	12/30[b]	8/30[a]	0/30[a]	0/30[a]	**16.6[a]%**
G3	0/30[a]	14/30[b]	3/30[a]	2/30 [a]	0/30[a]	0/30[a]	**0[a]%**
G4	5/30[b]	20/30[b]	25/30[b]	10/30[b]	2/30[b]	1/30[b]	**30[a]%**
G5	0/30[a]	7/30[a]	6/30[b]	5/30[a]	**0/30[a]**	0/30a	**3.3[a]%**
G6	12/30[b]	25/30[b]	25/30[b]	10/30[b]	5/30[b]	2/30[b]	**93.3[b]%**

[1]Challenge with VNDV genotype VII.1.1 (Chicken/USC/Egypt/2015), EID_{50} = 10^6 / 0.5 ml / bird via I/M route. G1 vaccinated by only HVT-ND, G2 vaccinated by HVT-ND and Live ND vaccine G3 vaccinated byHVT-ND, Live ND and Inactivated NDV vaccines , G4 that was vaccinated only with live ND vaccines G5 vaccinated by live ND and Inactivated NDV vaccines and G6 (non-vaccinated challenged).
a,b: different litters represent the statistical analysis significance of control and other vaccinated groups.

Table (4): Histopathological lesions in different vaccinated and control challenged groups on day 28 with VNDV genotype VII.I.I:

Lesion scores at dead birds and at 7 day post challange

Groups	Dead birds			7DPC		
	Trachea	Cerebellum	MSI	Trachea	Cerebellum	MSI
G1	3.25	2.[5 b]	2.8±*0.08*[b]	2	1	1.75±*0.08* [a]
G2	2.25[a]	2.5[a]	2.5±*0.02*	1 [a]	1[a]	1.6 ±*0.05*[b]
G3				1	1 [a]	1.18±*0.01* [a]
G4	3	2.75[a]	3 ±*0.5*[a]	1.5	2	1.65 ±*0.0*
G5	2	2	2±0.0[b]	1	0.5[a]	1.23±0.02[a]

G6	3.5[a]	4[a]	3.6±0.05[a]	2[a]	2.5[a]	2.5±0.05[a]

MSI: the formula of the mean severity indexis given by the function sum of mean lesions score.
a,b: represent the statistical analysis significance.

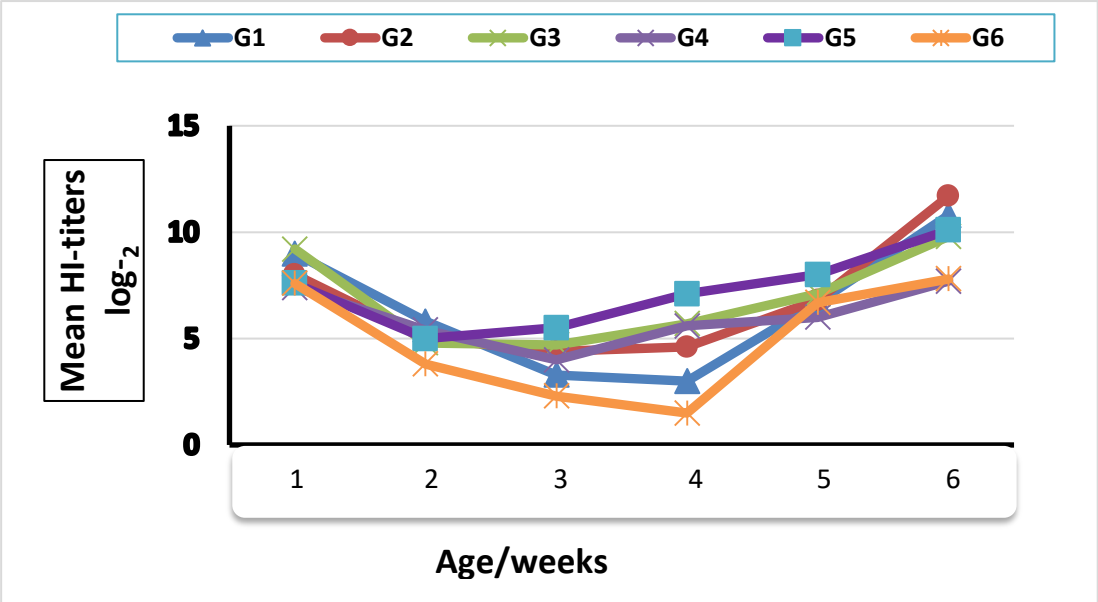

Fig (1). Mean HI titer log_2 in vaccinated and controlled challenged birds using NDV-genotype-VII Antigen.

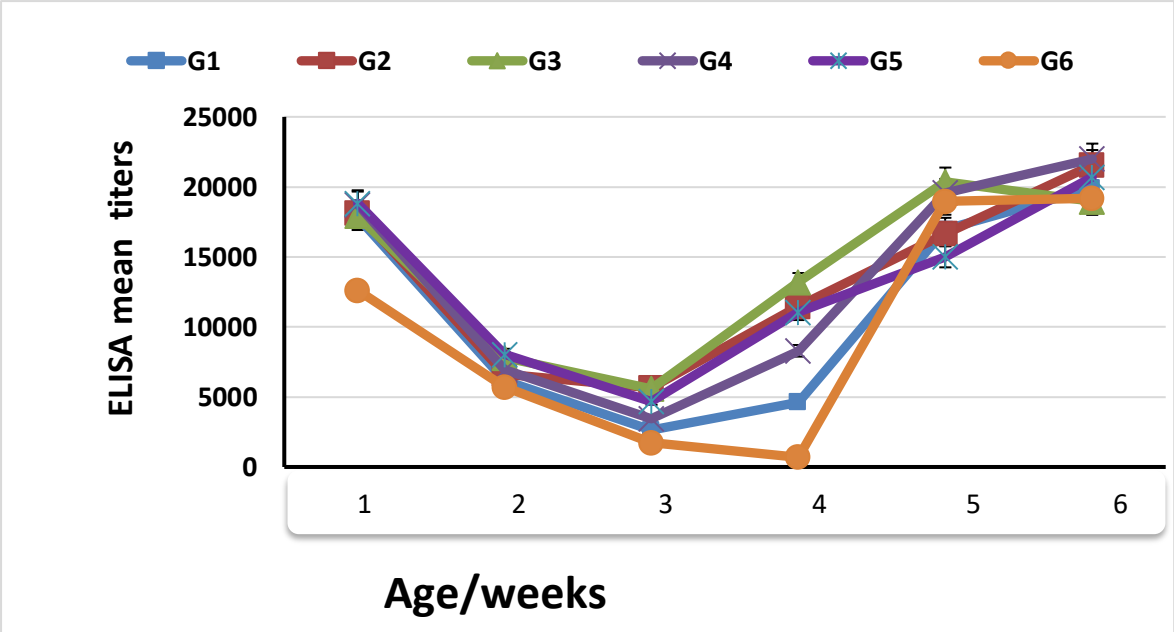

Fig. (2). Mean ELISA titer in vaccinated and non-vaccinated groups using NDV-genotype-VII Antigen.

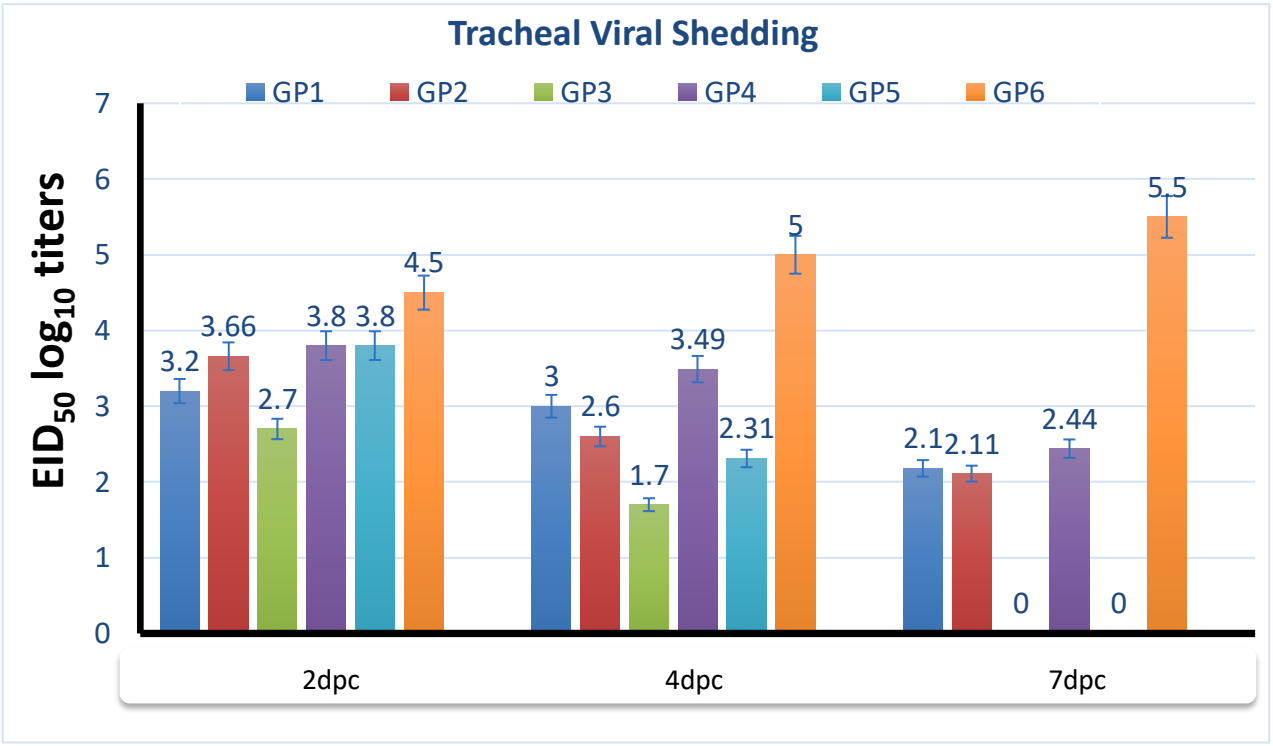

Fig. (3). Tracheal shedding titers log10 EID50/in broiler chickens vaccinated with different vaccination regimes.

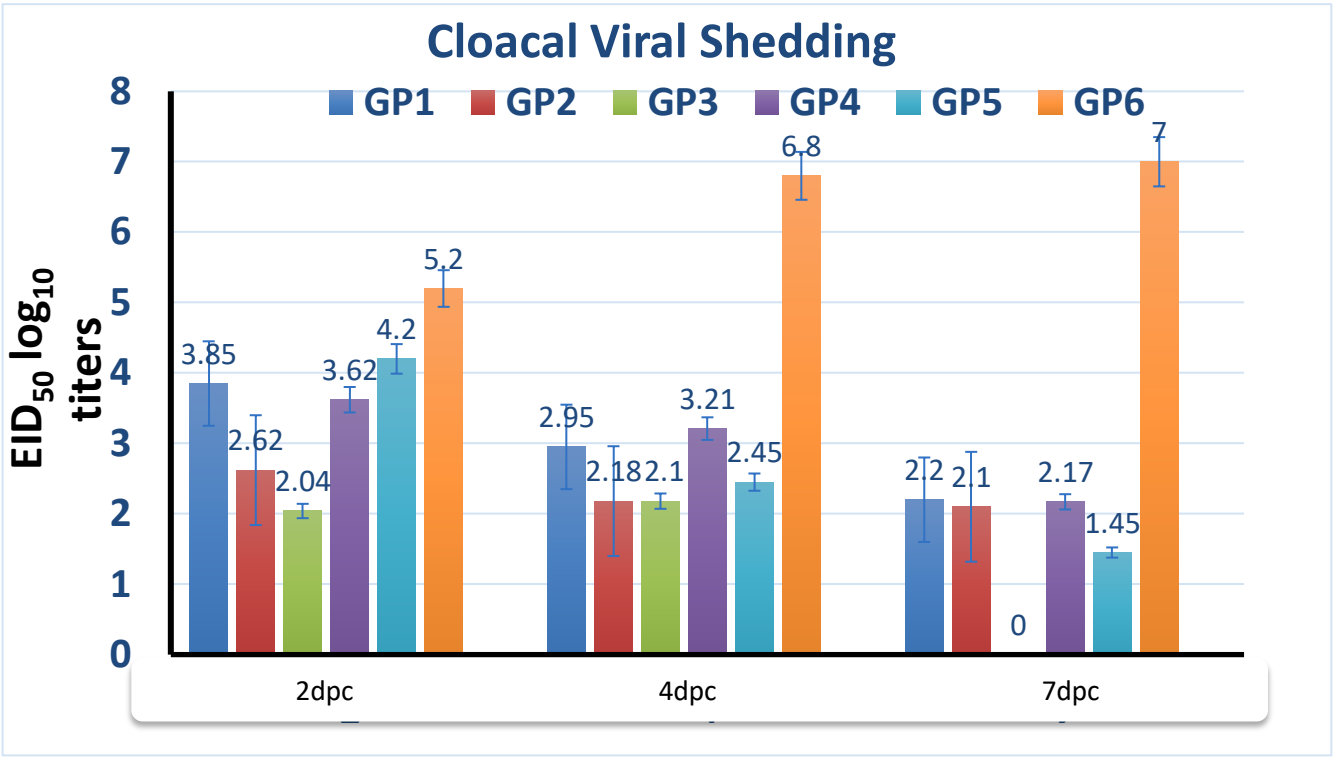

Fig. (3). Cloacal shedding titers log10 EID50/1ml in broiler chickens vaccinated with different vaccination regimes.

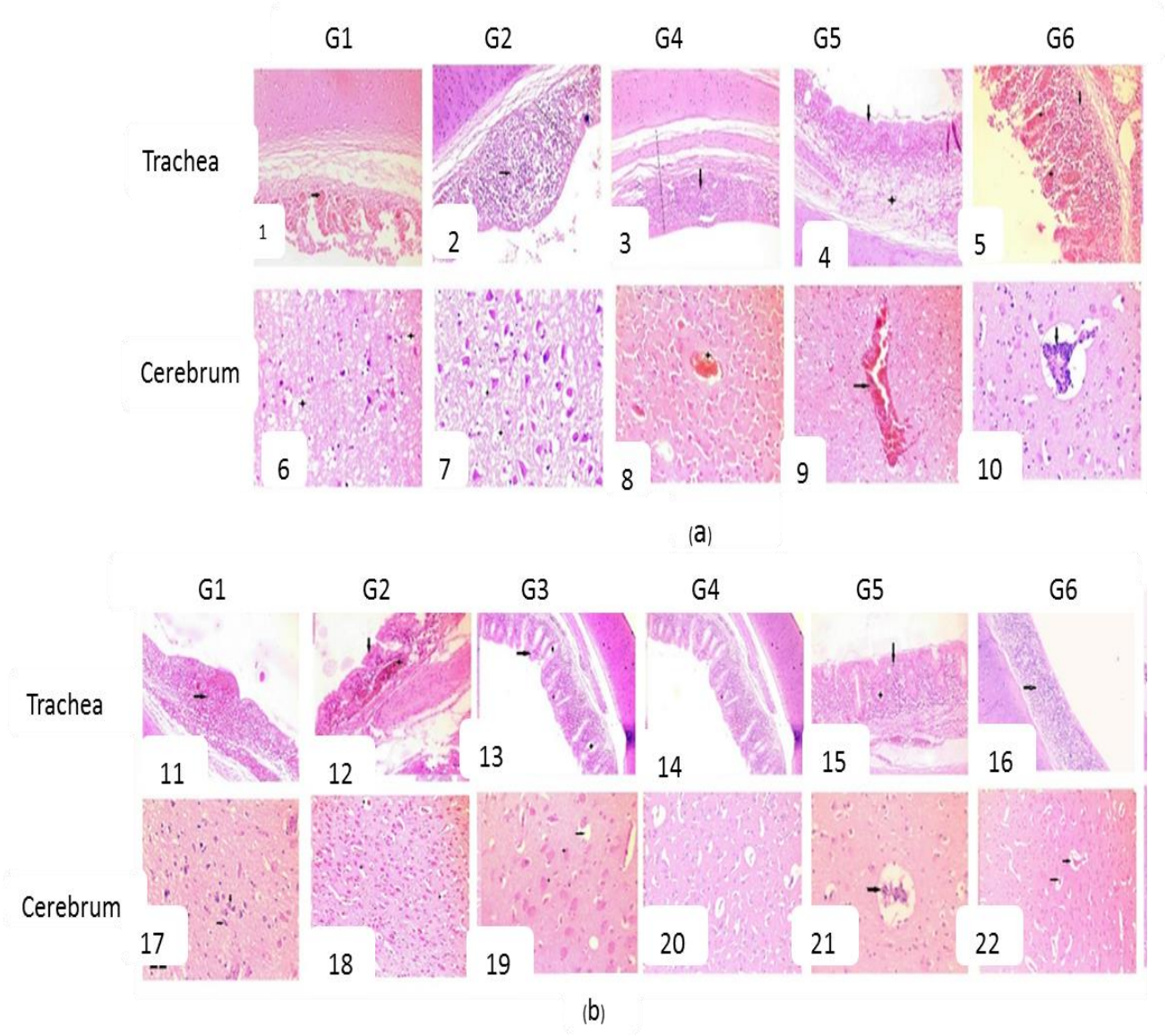

Fig. 5. (a). Histopathological changes of vaccinated challenged groups for dead birds G1, 1:Trachea demonstrated hyperplasia of epithelium lining with edema and blood vessels severe congestion (arrow) in lamina propria H&E X200. 6: Cerebrum show neutrophil vaculation (star) H&E X400 G2, 2: Trachea showing mucosa thickening (line), congested blood vessels, with edema and lamina propria has mononuclear cells infiltration (star) H&E X200.7: Cerebrum show neutrophil vacuolation and perineural edema (star) H&E X400. G4, 3: Trachea showing mucosa thicking (line), and edema with lamina propriamononuclear cells infiltration (arrow). 8: Cerebrum showing blood vessels congestion (star) G5, 4:Trachea show hyperplasia of epithelium lining (arrow) with edema of lamina propria (star) H&E X 200. 9: Cerebrum showed congestion at blood vessels (arrow) and perivascular edema H&E X200 G6:5:Trachea showing severe congestion at blood vessels (star), edema with inflammatory cells infiltration (arrow) H&E X200. 10: Cerebrum showing perivascular cuff, lymphocytes surrounded the blood vessels (arrow) H&E X400.

(b): Histopathological changes of vaccinated challenged groups 7 dpc .G1,11: Trachea showing thickening of the mucosa (arrow) with congested blood vessels, edema with mononuclear cells infiltration H& E X200. 17: Cerebrum showed slightly neurons degeneration with central

chromatolysis of few neurons (arrow) H&E X400. G2, 12: Trachea showing lining epithelium hyperplasia with edema and congestion of blood vessels and infiltration of inflammatory cells H&E X200. 18: Cerebrum showing slight perivascular edema. G3, 13: Trachea showing lining epithelium hyperplasia with edema, congestion and infiltration of inflammatory cells (arrow) H&E X200. 19: Cerebrum exhibits mild perivascular edema (arrow) and blood vessels congestion H&E X200.G4, 14: Trachea showing hyperplasia at lining epithelium and edema (arrow), congestion (star) and inflammatory cells infiltration H&E X200. 20: Cerebrum show slight perivascular edema (star) H&EX2009. G5, 15: Trachea showing lining epithelium hyperplasia (arrow) with mucous glands activation (star), edema, congestion with inflammatory cells infiltration at lamina propria H&E X200. 21: Cerebrum showing perivascular cuff (arrow) and neuropilvacuolation H&E X400. G6, 16: Trachea showing lining epithelium hyperplasia (arrow) with mucous glands activation H&E X100. 22: Cerebrum exhibits slight perivascular edema (star) H&E X200.

DISCUSSION

The extremely contagious viral disease NDV affects both domestic and wild birds, and it has a devastating financial impact on the production of poultry. Newcastle disease virus Genotype VII was first divided into two sub-genotypes VIIa and VIIb, which stood for viruses that originated in the Far East in the 1990s and moved to Europe, Asia and South Africa, while VIIc, d, and e isolates, which represent virus strains from China, Kazakhstan, and South Africa, respectively, and VIIf, g, h, and I which represent African isolates, are the subsequent sub-genotypes of VII (Alexander and Senne, 2008). The vast majority of recent reported outbreaks were located in the Middle East, Africa, and Asia (Khan et al., 2010). Due to mutations at various parts of F gene sites, including the N-glycosylation sites, epitope binding sites, and cysteine residues, which directly affect virus pathogenicity and thus interfere with the protection provided by conventional vaccines. More recent Egyptian NDV isolates were classified as genotype VII 1.1. (Dimitrov et al., 2019 and Selim et al., 2019). Live (lentogenic NDV strains) and inactivated vaccines are used to prevent Newcastle disease in order to create a strong immune response without having a significant negative impact on the birds (Dimitrov et al., 2017 and Meulemans et al., 1988). Despite immunization programs that rely on traditional outdated non-genotype-matched vaccines for the prevalent NDV in these endemic areas, ND outbreaks are common, with significant virus shedding and serious financial losses (Fawzy et al., 2021). Utilizing vector vaccines is a viable strategy for achieving the all following objectives, While live vector HVT/ND vaccine expressing F protein has been shown to induce both humoral and cell-mediated immunity in vaccinated chickens, protect chickens from lethal NDV challenge with genotype V viscerotropic velogenic strains, and reduce virus shedding after challenge, herpesvirus of turkeys (HVT) expressing foreign antigens related to poultry pathogens are considered to be the most potent vectors (Esaki et al., 2013). The control of ND still remains difficult, outbreaks are still occurring, despite the intensive vaccination programs applied to control ND (van Boven et al., 2008).

In this work, we examined the effectiveness of five different NDV immunization regimens, employing live and inactivated NDV vaccine with recombinant HVT-ND expressing F proteins in combination or alone against challenge of VNDV strain (Chicken/ USC/ Egypt/ 2015). Haemagglutination inhibition test results are considered one of the major assays to assess humoral response and the protection level conferred by NDV vaccines (Kapezynski and King, 2005). Vaccine uptake was monitored

for vaccinated broiler chickens in (Fig.1&2). All vaccinated groups elicited a significant increase in the immune response with a higher increase in G3 and G5 expressed as HI titer means log2 ± SE at 28 days of age compared with the control non-vaccinated groups which showed a gradual decrease in the HI titre means due to waning of maternal derived antibodies (MDA).After challenge, all challenged vaccinated birds exhibits an immune responsethat significantly rapid increase in the HI titer means at 35 and 42 days of age (Miller et al., 2013). The difference of HI titer can be explained by the role of the inactivated oil emulsion ND vaccine bykeeping the killed antigens at the injection site to release it gradually, causing local inflammation and stimulating the recruitment of antigen-presenting cells (APC), which then travel to the injection site to secondary lymphoid organs to interact with naive T and B lymphocytes in order to maintain a continuous high level of antibody production by repeated exposure of B cells to antigens or persistence of long-lived B cells (Rauw et al., 2014). On the other hand, the combination rHVT-ND/liveND vaccination schedule also led to greater immunity, which is consistent with(Ferreira et al., 2021). The ELISA NDV specific for F gene means titer were monitored. The non-vaccinated group showed high NDV ELISA titer at 7-days of age then declined at 28-days of age, while G3, G2and G5 showed higher ELISA titer at 28 day of age and lower titers were observed in G1 & G4 agreed with (Ferreira et al., 2021 and Esaki et al., 2013).

Post challenge severe clinical signs exhibit nervous manifestation including; tremors, torticollis and wing drop beside respiratory signs, greenish watery diarrhea, severe depression and off food, typical P.M gross lesion were observed for 5 dpc in G6 (non-vaccinated challenged) and mortalities started within 3 days post challenge reached 90% mortalities with the 7th day reflect the challenge virus's velogenic features and its pneumotropic, viscerotropic, and neurotropic affinities in the non-vaccinated birds. Versus no clinical signs nor mortalities in G3, while the other groups showed a varying degree of clinical signs and mortality 23.3 %, 13.3%, 33.3% and 3.3% mortality in G1, G2 ,G4 andG5, respectively. These results concluded that protection levels are directly proportional to the levels of humeral immunity gained from vaccination program, represented in the HI & ELISA means titers. we can conclude that G3 vaccination program provided the best protection in which we used combination of HVT-ND at 1 day of age, live NDVG-II at 1&14 days of age and inactivated NDV vaccine at 7 days of age, then G5 in which we used combination live NDVG-II at 1&14 and inactivated NDV vaccine at 7 days of age, these results were similar to Dortmans et al., (2012).Concerning protection achieved in G3, the result agreed with Charoenvisal et al. (2018) that concluded that all of the vaccination programs of rHVT-NDV vaccine could be used to reduce economic loss due to infection by Genotype VII NDV.

The MLS in dead birds were significantly reduced in G5 than other vaccinated groups while the more MLS was 3.5 in G6 with the same picture at 7dpc, the MLS were significantly reduced in G3and G5 comparing with other vaccinated groups, while the highest MLS was 2.5 in G6 (Non-vaccinated challenged). It was clearly shown that VNDV genotype VII have a great negative impact on birds immune system causing severe immunosuppression the same result reported by (Dimitrov et al., 2019 and Sultan et al., 2020). On comparing the histopathological lesions obtained from control non-vaccinated and other vaccinated groups it was clearly found that various NDV vaccines can minimize the effect of virulent NDV virus after challenge in the examined organs and produce significantly lower histopathological mean lesion scores (MLS).

The amount of NDV shed into the external environment differ according to vaccination

regimes used in our experiment. No viral shedding from tracheal swabs were detected from all groups at the day of challenge (0%) indicating that all groups didn't expose to field infection with virulent NDV. However after challenge, tracheal and cloacal shedding was significantly reduced in G3 and G5,while other vaccinated groups showed a higher level of viral shedding. Versus very high level of viral shedding in G6 (non-vaccinated challenged).Upon that, high protection level based on viral shedding was achieved in G3 vaccinated by combination of vector HVT-ND at 1 day of age, lives NDV at 1&14 and inactivated NDV vaccine at 7 days of age,before challenge, this group had significant antibody titers. The number of infected birds and the amount of vNDV shed decreased as humoral antibody levels rose in vaccinated birds as reported by (Perozo et al., 2012).Our findings were consistent with those of Palya et al. (2021), and Calderón et al. (2022) also produced a new recombinant virus called rHVT-F by using a Turkey herpesvirus (HVT) vector and expressing the fusion (F) protein of genotype XII and found similar results.

CONCLUSIONS

In conclusion, the best NDV vaccination program should focus not only on the prevention of clinical disease and mortality but also on decreasing virus shedding from vaccinated birds as an important consideration in endemic areas. The present study indicated that using the combination of vector HVT-ND, live, and inactivated ND vaccines as broilers NDV vaccination program reduced clinical signs, mortality, and postmortem manifestations in addition to tracheal and cloacalvirus shedding against challenge with heterologous NDV genotype VII.1.1.

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