Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Avian Diseases

Efficacy of the Genotype-Matched and Non-Matched Vaccines Against Newcastle Disease Virus Genotype VII.1.1. Challenge in Broiler Chickens

Elsayed Kamel[1*] and Hesham Sultan[2]

(1) Veterinarian at the Directorate of Veterinary Medicine, Menoufia, Egypt.
(2) Department of Avian diseases, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

**Corresponding author:* elsayedghram@gmail.com *Received: 1/10/2022 Accepted: 29/10/2022*

ABSTRACT

Despite extensive vaccinations attempts, genotype VII Newcastle disease viruses (NDV) continue to circulate at the Middle East and Asia causing significant economic losses. In this investigation, we assessed the effectiveness of three inactivated NDV vaccines; genotype II NDV, recombinant genotype VII NDV-matched and laboratory prepared autogenous velogenic NDV genotype VII against challenge by genotype VII velogenic NDV. The following vaccinations were given: group 1(G1) received an inactivated genotype II vaccine, group 2 (G2) received an inactivated recombinant genotype VII NDV-matched vaccine, and group3 (G3) received a velogenic inactivated autogenous NDV genotype VII vaccine. While the live genotype II (Lasota) vaccine was administered to all groups. At the age of 28 days, birds in all vaccination groups were challenged with NDV genotype VII, and protection against infection was assessed based on the mortality number, clinical symptoms, Post mortem lesions, virus shedding, and histological changes. The results of clinical protection were78%, 87% and 96% in vaccinated groups G1, G2 and G3 respectively, against 7 % in the control non-vaccinated challenged group. Virus shedding in all vaccinated groups significantly reduced when compared to the non-vaccinated group (G4). The mean lesion score on 4 and 7 days post challenge were significantly reduced in G3 and G2 compared to G1.In conclusion, more protection against challenge was offered by a velogenic inactivated autogenous NDV genotype VII vaccine that was closely related to genotype VII.1.1 strain than by other vaccines. So we recommended the use of genotype matched vaccine to control NDV in endemic countries.

Keywords: Commercial broiler, Genotype-matched vaccine, Genotype VII.1.1 and Newcastle disease virus.

INTRODUCTION

Virus infections that affect the poultry raising including one of the more prevalent and contagious diseases is Newcastle disease (NDV) (Hines et al., 2012). Disease is mainly caused by a group of viruses that is related to genus Orthoavulavirus, Avian orthoavulavirus1 species (Dimitrov et al., 2019). They are typically referred to as Newcastle disease viruses or Avian paramyxovirus 1 (APMV1). It has been noted during the past few decades that there has been a significant change in the virulence of the velogenic NDV (vNDV) strains that are known to be common in poultry (Hu et al., 2012). According to phylogenetic analysis recent virulent isolates were identified as being closely related to virulent strains that were first detected in the 1940s and have since been utilized repeatedly in experimental challenge trials (Miller and Koch, 2013). NDVs genome is negative sense single

stranded, non-segmented RNA that mainly encode for at least six structural proteins. Class II viruses are categorized into 18 genotypes (I-XVIII) based on analysis of the nucleotide sequence of the F gene, with genotypes V, VII, and VIII being the most prevalent genotypes circulating globally (Alexander and Senne, 2008). The genotype VII landscape has been changed significantly by using the new classification criteria (Dimitrov et al., 2019). All NDV genotypes share antigenic similarities, and as a result, all viruses will cross-protect against challenge from one another. Therefore, it was established that immunizations with live low virulence NDV vaccines can actually protect the birds against vNDV (Alexander, 1988 and Diel et al., 2012). The first diagnosis of NDV in Egypt was as early as 1948 (Daubney and Mansy, 1948). While NDV sub-genotype VIId was considered as the predominant strain that has led to several outbreaks in poultry since it was firstly isolated in Egypt (Radwan et al., 2013). According to more recent classification, the more recent Egyptian NDV isolates are related to genotype VII 1.1 as a result of mutations at various F gene sites, these sites are the N-glycosylation sites, binding epitopes sites, and cysteine residues, which primarily affect virus pathogenicity and may interfere with the protection provided by using conventional vaccines (Hines et al., 2012). Many countries in all over worlds have continuously experimented newer NDV vaccines. These innovative vaccinations strategies are mostly based on recombinant herpes virus-vectored that express velogenic F and/or HN genes and reverse genetic LaSota NDV vaccines (Li et al., 2016). Egypt now provides two different types of vaccines for NDV (genotype II and the most recent genotype VII), which are mostly used to evaluate protection offered by them and shows the amount of virus shedding that contaminates the environment following challenge with VVND-GVII.1.1 (Miller et al., 2009 and Miller et al., 2007). Recombinant GVII-matched chimeric vaccines have recently been established, as the Himmvac Dalgoban N (Plus) Oil Vaccine, which provided notable protection in specific pathogen-free (SPF) chicken against infection with the homologous genotype NDV. The primary objective of our study was to assess the effectiveness of the inactivated Genotype II (LaSota) and inactivated recombinant reverse genetic ND vaccine LaSota backbone that carrying F-HN genes from NDV-GVII isolate in Asia and autogenous laboratory prepared VNDV genotype VII.I.I inactivated vaccine against challenge with NDV GVII 1.1 in commercial broiler chickens.

MATERIALS AND METHODS

Vaccines:

Vaccination regimes included inactivated oil emulsion genotype II NDV vaccine against Newcastle disease virus, vaccinal dose contain ND Clone 30 ≥ 50 PD_{50} obtained from MSD, Spain, administrated by a dose of 0.5 ml through S/C.; an inactivated oil emulsion vaccine prepared by reverse genetic reassortment where the virus has been prepared by the deletion of the multi-basic cleavage sites at the F gene as away of attenuation then it was inserted in the backbone of Lasota vaccine as previously described with a concentration of 8.2 log_{10} EID_{50} (OIE, 2012), and an inactivated oil emulsion autogenous genotype VII 1.1 NDV vaccine with a concentration of 8.2 log_{10} EID_{50}. This vaccine was prepared in our study as a whole inactivated VVND genotype VII 1.1 vaccine where the antigen propagated in SPF embryonated chicken eggs (ECE), followed by antigen harvesting and inactivation then mixed with oil adjuvant (Al-Habeeb et al., 2013).

Challenge virus:

The challenge virus used in this investigation was previously identified and characterized (NDV Chicken/USC/Egypt/El Gharbia/2015), and its genetic structure matched that of the

autogenous vaccine that had been laboratory prepared (Sultan et al., 2020).

Experimental Design*:*

One hundred eighty day-old broiler chicks having passive maternal NDV anti-bodies from a local breeder flock that had been previously vaccinated with inactivated NDV genotype II vaccines four times (on days 15, 50, 90, and 125 of life) and five times with live ND genotype II vaccines (on days 9, 42, 80, 120, and 150 of life). The chicks were divided randomly into 4 groups (G1 to G4), each with 45 birds. Under the same guidelines, they were kept in separate enclosures with free access to food and water at a biosecured experimental facility. We chose a common routine immunization program for broilers in Egypt that included two doses of a live ND Lasota vaccine (at 7 and 17 days of age was administrated by eye drops) and one dose of an inactivated oil emulsion ND vaccine on the age of 7 day for G1, G2, and G3 (Table 1). All birds in all groups were challenged with the virulent NDV, USC2015 strain (10^6 EID_{50} / bird) through the intranasal route. Before the challenge, cloacal and tracheal swaps from all groups (10 birds per group) were collected, and they were examined utilizing primer sets for real-time reverse transcriptase polymerase chain reaction (RT-PCR) against common NDV (Leary et al., 2013). For about 10 dpc, clinical indications parameters in every experimental group were watched, recorded. Swab samples from the trachea and cloaca were taken separately at 2, 4, and 7 dpc to measure the amount of viral shedding.

Serology:

According to the OIE Manual, serum antibodies against Newcastle disease were measured using the hemagglutination inhibition (HI) assay . On days 7, 14, 21, 28, 35, and 42 of life, blood samples were taken. then serum separated and kept at -20 °C until testing . Four hemagglutination units (4HAU) each of the NDV LaSota and NDV-genotype VII antigens were used in the assays. HI titers means were represented as log2 values, and samples with HI titers equal to or more than 4 log2 were regarded to be positive as described before (Al-Habeeb et al., 2020).

Histopathological Examination:

Brain and trachea tissue samples were taken, fixed in 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 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, 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 (Bancroft and Stevens. 1977).

Virus shedding:

Tracheal and cloacal swabs from 10 distinct birds/group were taken at 2, 4, and 7 dpc and placed in 400 µL of Dulbecco's modified Eagle medium with an antibiotic solution. Thenviral RNA was directly extracted using the TRIzol reagent (Gibco, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Using specific primer and probe sets from (QIAGEN, Valencia, CA, USA), standard RT-PCR was carried out. The QuantiTect RT-PCR Master Mix, 0.25 M L 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. A 30-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

convertedintolog$_{10}$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:

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 using Student's t-test or ANOVA, then Duncan's new multiple range test is applied (Steel and Torrie, 1960).

RESULTS

Clinical signs, Gross lesion and Mortalities:

Concerning the G-1, 64% (29/45) of the birds had greenish diarrhea but no ocular lesions, and 46% (21/45) of the birds displayed mild or severe respiratory symptoms (nasal discharge, respiratory rales, lacrimation and slight occlusion to eye). After the recovery stage, only 8% (4/45) of patients exhibited nerve symptoms (paresis), and death was 22% (10/45) at 10 dpc. While in the G-2, 35% (16/45) of birds displayed mild respiratory symptoms and nasal discharge, 51% (23/45) displayed greenish diarrhoea, and 2% (1/45) displayed ocular abnormalities (lacrimation, slight occlusion to eye). The mortality rate was 13% (6/45) at 10 dpc. While, birds in G-3 showed the higher protections rate with minor respiratory symptoms and nasal discharge and 22% (10/45) of the birds had greenish diarrhea but no ocular abnormalities, and the majority of them recovered fast with 96% (2/45) clinical protection within 10 dpc and only 4% (2/45) mortality at 10 dpc. On the other hand the picture differ significantly in G-4 where 100% (45/45) of the birds displayed moderate to severe respiratory symptoms, 91.4% (40/45) of the birds experienced greenish diarrhea, 13% (6/45) of the birds displayed neurological manifestations and paresis, and 93% (42/45) of the birds died, with only 7% (3/45) of the birds making a full recovery (Table, 2). P.M examination showed that G1, G2, and G3 dead birds had minor or no proventriculus petechial hemorrhages at tip of glands, whereas G4 dead birds have severe petechial hemorrhages. In addition, the spleens of the birds from groups G1, G2, and G3 showed mild to moderate focal necrosis, whereas the birds from group G4 had very severe focal necrosis in the spleen. Groups G1, G2, and G3 showed mild or moderate catarrhal tracheitis and lung congestion whereas severe catarrhal tracheitis was demonstrated in group4 (G4). The G1, G2, and G3 birds had mild cecal ulcers, whereas the G4 birds had moderate cecal ulcers.

Serum antibody response to vaccination:

Regardless of the genotypes of the inactivated NDV vaccines, the results demonstrated that there were only small, statistically significant differences between the various vaccinated groups. According to data from the age of 28 days, HI titers utilizing GII antigen were 4.3 in G1 and G2 but 4.8 in G3 against 0.2 in G4 the non-vaccinated group log2 (Fig. 1). The mean HI titers of the groups before and after the NDV challenge were shown in (Fig.1 & 2).

Histopathology:

In (G4), severe lesions were evidently seen in all tested organs (trachea and cerebrum) after 28 days. Samples were taken from various organs at 4 and 7 dpc, including the trachea and cerebrum, where the main lesion scores were 2.4, 2.1, 1.7, and 3.4 on the 4th dpc in G1, G2, G3, and G4, respectively, and were 1.98, 1.6, 1.25, and 3.5 on the 7th dpc, as shown in (Table 3). Microscopic examinations of birds in G4 in all organs that were tested revealed very severe histopathological lesion scores. Lesions scored substantially higher than those of birds in groups that had received vaccinations. Broilers who received inactivated genotype II vaccine (G-1) showed

substantial histological lesions in the trachea and cerebrum compared to the lower lesions score in G-2 and G-3(vaccinated by genotype VII) (Fig. 3).

Virus Shedding:

Viral shedding results showed that virus titers in tracheal and cloacal swabs also numbers of shedder birds in G4 were significantly higher compared to G1, G2 and G3 as illustrated in (Table 4).

Table (1): Experimental grouping, used vaccines and vaccination regimes as well as assessment of immune response in commercial broiler chickens:

Group No.	Birds No.	NDV vaccination regime		Challenge[1] Age/day	Assessment of protection
		Vaccine genotype	age/days		
1	35	Live G-II [2] Inactivated [3]NDV-GII	7, 17 7	32	**1.** Clinical signs **2.** Pm. Gross lesions **3.** Mortality% **4.** Sero-conversion **5.** Detection of viral shedding **6.** Histopathology
2	35	Live G-II Inactivated [4]rNDV-GVII	7, 17 7		
3	35	Live G-II Inactivated[5]Auto-NDV-GVII	7, 17 7		
4	35	Non-Vaccinated challenged			

[1]Challenge with VNDV genotype VII.1.1 (Chicken/USC/Egypt/2015), $EID_{50} = 10^6$ / 0.5 ml / bird via occulo nasal route

[2]Live LaSota NDV strain vaccine, of genotype II, Each dose/ bird contains ≥ 5.5 $\log_{10}$ EID_{50}. administrated via eye drop

[3]inactivated LaSota genotype II vaccine with dose equal 8.2 $\log_{10}$ EID_{50} administrated 0.5 ml by S/C rout.

[4]inactivated recombinant-LaSota GVII vaccine with dose equal 8.2 $\log_{10}$ EID_{50} administrated 0.5 ml by S/C rout.

[5]inactivated autogenous experimental prepared GVII vaccine with dose equal 8.2 $\log_{10}$ EID_{50} administrated 0.5 ml by S/C rout.

no = Number.

Table (2): Mortality and clinical signs in vaccinated and control challenged groups on the age of 28 day with VNDV genotype VII.I.I:

Groups	Vaccine Genotype	Occular Lesion	Respiratory Symptoms	Diarrhea	Nasal Discharge	Paresis	Nervous signs	Mortality. (%)
G1	NDV-GII	$0/45^a$	$16/45^b$	$29/45^b$	$5/45^a$	2/45	2/45	**22 %**
G2	rNDV-GVII	$1/45^a$	$12/45^b$	$23/45^b$	$4/45^a$	0/45	0/45	**13%**
G3	Auto-NDV-GVII	$0/45^a$	$5/45^a$	$10/45^a$	$2/45^a$	0/45	0/45	**4%**

G4	Non-Vac	20/45[b]	39/45[b]	40/45[b]	22/45[b]	4/45	2/45	**93%**

G1, Inactivated Genotype II, lasota. G2, Inactivated recombinant GVII. G3, Inactivated autogenous GVII. G4, Control group. a, b: represent the statistical analysis significance between control and other vaccinated groups.

Table (3): Histopathological lesions in different vaccinated and control challenged groups on day 28 with VNDV genotype VII.I.I:

Lesion scores at DPC

Groups	4DPC			7DPC		
	Trachea	Cerebellum	MSI	Trachea	Cerebellum	MSI
G1	2	2[b]	2.4±*0.78*	2.3	2.1	1.98±*0.28*[a]
G2	2[a]	2	2.1±*0.23*	1.9[a]	1.5	1.6 ±*0.17*[b]
G3	1.9[b]	1.9[a]	1.7 ±*0.12*	2	1.3[a]	1.25±*0.18*[a]
G4	3.4	3.4	3.4 ±*0.5*	3.6	3.4	3.5 ±*0.0*

MSI: The mean severity indexis calculated by summation of mean lesions score function.
Mean within the same columns are significantly differ at ($p \leq 0.05$).

Table (4): VV-NDV viral shedding in different broiler chickens challenged groups:

Viral shedding at dpc

Group no.	**0**		**2 dpc**		**4 dpc**		**7 dpc**	
	Tr	**Cl**	**Tr**	**Cl**	**Tr**	**Cl**	**Tr**	**Cl**
G1	**0/10***	**0/10**	**4/10 6.5±1.2[a]****	**4/10 6±1.4[a]**	**4/10 4.5±2.3[a]**	**4/10 4.5±1.32[a]**	**0/10**	**0/10**
G2	**0/10**	**0/10**	**2/10 2.3±0.0[a]**	**2/10 4.5±0.0[a]**	**2/10 1.4±0.0[a]**	**4/10 3.5±1.3[a]**	**0/10**	**0/10**
G3	**0/10**	**0/10**	**2/10 2.1±0.0[a]**	**2/10 2±0.1[a]**	**2/10 1±0.1[a]**	**2/10 3±0.2[a]**	**0/10**	**0/10**
G4	**0/10**	**0/10**	**6/10 8.5±1.4[b]**	**6/10 8±0.8[b]**	**10/10 9±1.76[b]**	**10/10 7±1.31[b]**	**9/10 7.8 ± 0.87[a]**	**9/10 8±0.6[b]**

*Positive no./Examined no.
**NDV titer Log_{10} EID_{50}/1 ml
Means within the same column of different litter are significantly differ at ($P \leq 0.05$).
dpc: day post challenge
Tr: Tracheal

Cl: Cloacal

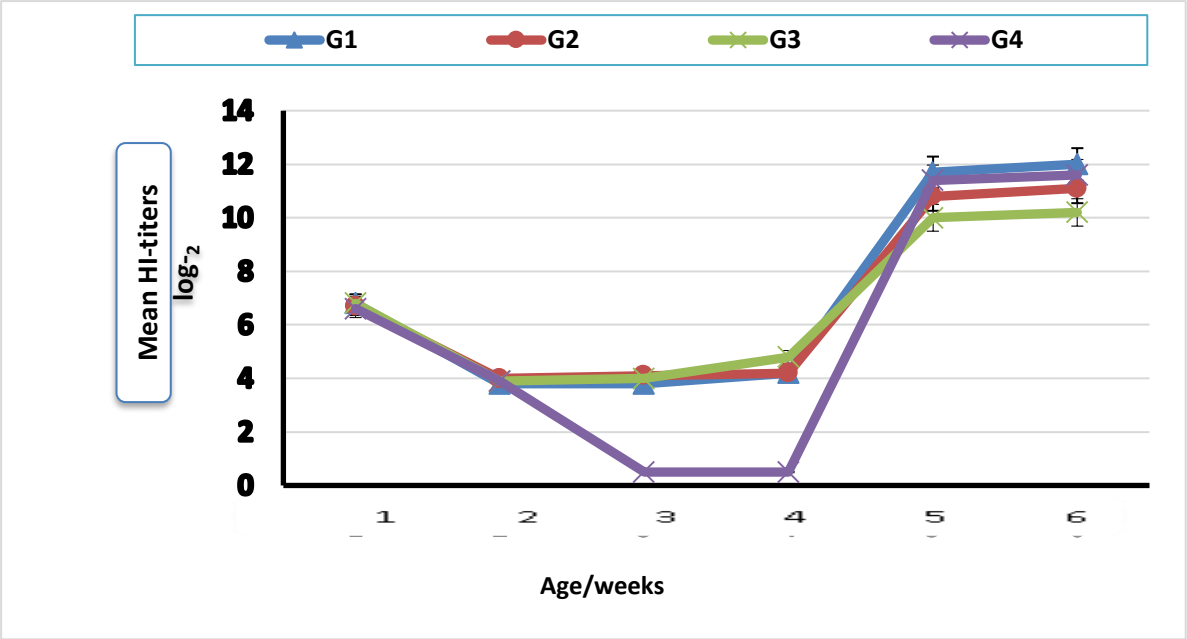

Fig. (1). Mean HI titer log_2 in vaccinated and controlled challenged birds using LaSota Antigen.

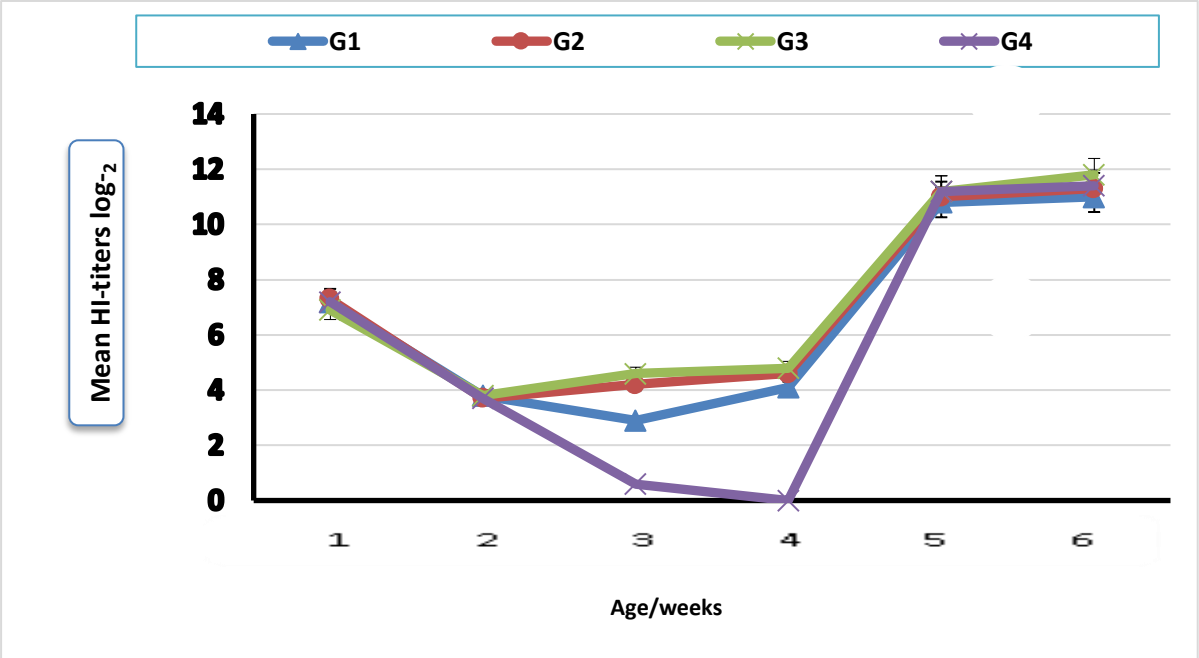

Fig. (2). Mean HI titer log_2 in vaccinated and controlled challenged birds using NDV-genotype-VII antigen

.

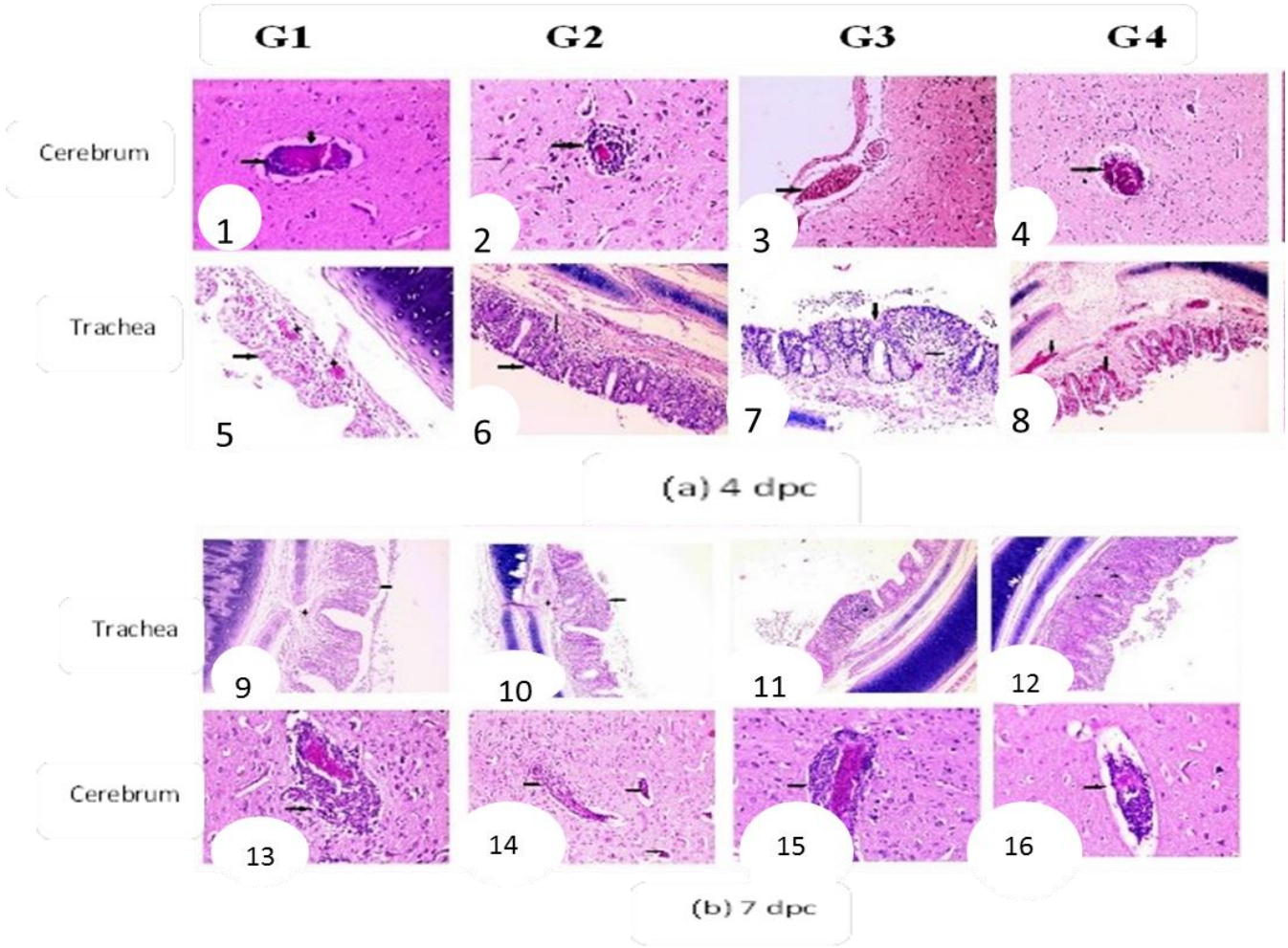

Fig. (3). a.1: Cerebrum (G1) demonstrating lymphocytic perivascular cuffs (arrow)(H&E 400×). 2: Cerebrum (G2) showing lymphocytic perivascular cuffs with perivascular edema (arrow) and neuronal degeneration (line) (400×). 3: Cerebrum (G3) showing congested blood vessels sub-meningeal (200×). 4: Cerebrum (G-4) showing perivascular cuff, large, and small blood vessels surrounded by inflammatory cells (200×). 5: Trachea (G1) showing hyperplasia of underling epithelium (arrow) and extensive mucosal and submucosal congestion and edema (star) (H&E 100×). 6: Trachea (G-2) showed hyperplasia of epithelium lining (thick arrow) with mild congested blood vessels, edema activation of mucous glands, and few mononuclear cells infiltration in lamina propria (thin arrow) (200×). 7: Trachea (G3) showing hyperplasia of lining epithelium and edema, mucous glands activation (thick arrow), with few mononuclear cells infiltration in lamina propria (thin arrow) (H&E 200×). 8: Trachea (G4) edema with severe lamina propria congestion (arrow) (H&E 400×). **(b):** 9: Trachea (G1) showing hyperplasia of lining epithelium (arrow) (H&E 200×). 10: Trachea (G2) showing hyperplasia of lining epithelium (arrow) with edema and lamina propria congestion (H&E 100×). 11: Trachea (G3) showing hyperplasia oflining epithelium, thickening of mucosa(line), and lymphocytic nodule (star) and mucosal and submucosal edema (H&E 200×). 12:Trachea (G-4) 7dpc showing thickening of mucosal layer (arrow), edema, congestion of blood vessels, and mononuclear cell infiltration with active mucous gland (H&E 100×). 13: Cerebrum (G-1) blood vessels congestion and perivascular cuff (arrow) (H&E 400×). 14: Cerebrum (G-2) demonstrating multiple perivascular cuff (arrows) (H&E 400×). 15: Cerebrum (G-3) showing many perivascular cuff (arrows) (H&E 400×). 16: Cerebrum (G 4) show lymphocytic perivascular cuff (arrow) (H&E 400×).

DISCUSSION

ND is an economically severe devastating viral disease that affects the poultry industry (Bello et al., 2018). NDV-GVII has been associated with the most of recently occurring outbreaks (Khan et al., 2010 and Rehmani et al., 2015). Despite the use of many vaccination strategies depending on conventional or non-genotype-matched vaccines, numerous ND outbreaks with increasing viral shedding and significant economic losses by deaths or decreased egg production are still occurring (Fawzy et al., 2020).

The birds in the non-vaccinated challenged groups (G-4) showed almost 100% respiratory manifestations, 91.4% digestive disorders, and only 13% of the group recovered, showing later nervous manifestations and a 91.4% mortality rate. In this study, protection against the development of clinical manifestations was significantly higher in the vaccinated challenged groups than in the non-vaccinated challenged birds (Table 2), reflecting the characteristics velogenic, viscerotropic, pneumotropic and neurotropic affinities of the challenge virus in non-vaccinated chicks (Ayoub et al., 2019 and Moharam et al., 2019). In G-1 (genotypeII inactivated vaccine) birds showed the most respiratory manifestations and digestive signs (46% and 64%, respectively) and 8% of birds showed nervous signs with a low recovering bird as reported by Fawzy et al. (2020). However, compared to the birds in G-1, the birds in G-2 (vaccinated with recombinant G-VII and Challenged) had reduced incidences of respiratory and digestive symptoms (35% and 51%, respectively). Data from the birds in G-3 (received autogenous NDV-GVII) revealed that clinical protection against developing clinical manifestations is improved by increasing homology and a close matching degree between the challenge NDV and the vaccine seed. The birds in G-3 showed the lowest rates of respiratory and digestive manifestations at 11% and 22%, respectively, as previously reported by (Moharam et al., 2019 and Miller et al., 2007).

Concerning gross lesions, vaccinated groups (G1, G2 and G3) exhibits mild lesion, while G4 revealed the recognizable, usual gross lesions of NDV in the dead chickens such as ulceration patches in the cecal tonsils, petechial haemorrhages in the tips of proventriculus, intestinal button like ulcers congested hemorrhagic tracheitis, and pneumonia. It was shown that the severity of the gross lesions decreased from the autogenous NDV vaccinations to the recombinant G-VII vaccines to the Genotype II vaccines as the level of matching between the applied vaccines and the challenge virus increased. Similar to this, Sedeik et al. (2018) found that groups that had received the genotype-matched NDV vaccine (NDV GVII-origin vaccine) had significantly higher levels of protection against the clinical signs of ND, the postmortem gross lesions, and the mortality rate than groups that had received the non-genotype-matched inactivated NDV GII-origin vaccine when challenged with velogenic NDV GVII.

Consequently, the birds were serologically monitored before being infected during an experiment, titers of HI were 4.3 in G-1 and G-2 but it was more in G3 4.9 log2, as shown in (Fig. 1&2), indicated that greater humoral immune responses can be mounted with combining repeated doses of live and inactivated NDV vaccines. The immune response from the age of 14 until the age of 21days of life showed variable statics levels, with cell-mediated immunity being stronger than humoral immunity due to a live booster vaccine given on the age of 17 day. Additionally, the humoral immunity obtained by vaccine helped the chicks with high MDA levels to make up for the declining of those levels by the third week of life (Ayoub et al.,

2019). At the level of humoral immune response, there were no discernible changes between the three vaccination groups (G1, G2, and G3). In addition, the chicks that had been enhatched with high MDA benefited from the humoral immunity obtained from the vaccines, which compensated from the waning in the MDA at the 3rd week of the age (Ayoub et al., 2019). The humoral immune response by vaccinal takes (based on the HI assay) did not differ significantly between the three vaccine groups (G1, G2, and G3), but there were differences in the levels of protection against the emergence of clinical manifestations and protection against fatalities (78%, 87%, and 96% in G1, G2, and G3, respectively). By using NDV-genotype VII antigen the higher immune response was showed in the groups that received the NDV genotype VII vaccine (G2 and G3) than the other received genotype II inactivated vaccine (G1).In the same contexts Sultan et al. (2020), reported that the level of clinical protection was variable between various genotypes of inactivated ND vaccines although they may have a nearly similar levels of HI means titers. We can see how important it is for a highly specific humoral immune response to control the ND virus and come to the conclusion that because ND vaccine genotypes vary and some circulating viruses do not match vaccines, higher HI titers may be required, which can be obtained from multiple doses of the non-genotype-matched vaccine, whereas a genotype-matched vaccine only needed a smaller amount of titers to provide protection (Miller et al., 2007). Due to the difference in F-gene epitope sites between the previous NDV vaccines and the now prevalent NDV virus the protection against mortality will be variable (Liu et al., 2017).

In broiler chickens, the pathogenesis of VVNDV G-VII.1.1 reveals microscopic alterations (Fig. 3) in many internal organs and reflects the levels of protection provided by vaccination. The challenged non-vaccinated birds (G-4) histological investigations exhibited severe pathological lesions in the tested organs, where the trachea displayed significant edema, extensive lamina propria congestion, and tracheal mucosa sloughing. Inflammatory cells encircled both big and small blood vessels, and the cerebral cortex showed a perivascular cuff (Palya et al., 2012). When compared to birds that had received both inactivated genotype VII vaccines (G2and G3), the birds in G-1 who had received the genotype II inactivated vaccines at 7 (dpc) we noted higher lesion scores in G1 (1.98± 0.28) in comparison to (G2 and G3),with lesion scores of 1.6±0.17 and 1.25 ±0.18 , respectively. In the same manner the results at 4 dpc reveal that the birds in G-1 havehigher score lesions compared to birds in (G2 and G3).This observation, which is in agreement with Sultan et al., (2020) may explain the decreased fatality rates in G-2 and G-3 compared to G-1 by stressing the significance of genotype-matched vaccinations in the protection against VVNDV challenge.

The results of the RRT-PCR used to measure virus shedding (Table 4) revealed that the non-vaccinated challenge group (G-4) showed very high virus shedders (10/10) at 2, 4, and 7 dpc, confirming the velogenicity of the challenge virus. G1, G2 and G3 dramatically decreased virus shedding in terms of both number of shedders and the amount of virus that will be shed, in contrast to other non-vaccinated groups (Moharam et al., 2019). In accordance with earlier results by Fawzy et al., (2020) the genotype-matched vaccine (G-2 and G3) resulted in a greater reduction of shedding in birds than the genotype II inactivated vaccine (G1).

CONCLUSIONS

Genotype-matched inactivated NDV vaccines (recombinant GVII or autogenous GVII) revealed more clinical protection based on

mortality rate, severity of microscopic changes and significant reduction in NDV shedding than genotype-mismatched (GII) in commercial broilers that were challenged with velogenic NDV-GVII infection. Additionally, the new mutation that has been seen in the F-gene can be covered by the use of a closely matched NDV vaccine, which may be related to higher survival rates than usually provided by old conventional one. In order to ensure effective and profitable strategies for control NDV in broiler chickens, it is also necessary to assess the effectiveness of the available commercial vaccines that are currently up against the challenge with the recently developing GVII in the poultry field in Egypt.

REFERENCES

Alexander, D and Senne, D. 2008. Newcastle disease and other paramyxoviruses. In A Laboratory Manual for the Isolation Identification and Characterization of Avian Pathogens, 5th ed.; Dufour-Zavala, L., Swayne, D.E., Pearson, J.E., Reed, W.M., Jackwood, M.W., Woolcock, P., Rglission, J.R., Eds.; OmniPress, Inc.: Norristown, PA, USA, pp. 135–141.

Alexander, D.J. (1988). Newcastle disease diagnosis. In Newcastle Disease; Springer: Cham, Switzerland, pp. 147–160.

Al-Habeeb, M. A., Mohamed, M. and Sharawi, S. 2013. Detection and characterization of Newcastle disease virus in clinical samples using real time RT-PCR and melting curve analysis based on matrix and fusion genes amplification. World 2013, 6, 239–243.

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, 197–206.

Bancroft, J. D. and Stevens, A. 1977: Theory and Practices of Histologic Techniques. Churchill, Living Stone Edinburgh, London, New York 2nd Eds., ISBN, 044304760X.

Bello, M. B., Yusoff, K. M., Ideris, A., Hair-Bejo, M., Peeters, B. P. H., Jibril, A. H., Tambuwal, F. M. and Omar, A. R. 2018. Genotype Diversity of Newcastle Disease Virus in Nigeria: Disease Control Challenges and Future Outlook. Adv. Virol., 2018.

Daubney R. and Mansy, W. 1948. The occurrence of Newcastle disease in Egypt. J. Comp. Pathol. Therapeut., 58: 189–200. https://doi.org/10.1016/S0368-1742(48)80019-6

Diel, D. G., da Silva, L. H., Liu, H., Wang, Z., Miller, P. J. and Afonso, C. L. 2012. Genetic diversity of avian paramyxovirus type 1: Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infect. Genet. Evol. 12, 1770-1779.

Dimitrov, K. M., Abolnik, C., Afonso, C. L., Albina, E., Bahl, J., Berg, M…Wong, F. Y. K. 2019a. Updated unified phylogenetic classification system and revised nomenclature for New castle disease virus. Infect. Genet. Evol., 74: 103917.

Fawzy, M., Ali, R., Elfeil, W., Saleh, A. and Eltarabilli, M. 2020. Efficacy of inactivated velogenic Newcastle disease virus genotype VII vaccine in broiler chickens. Vet. Res. Forum ,11, 113–120.

Gibson-Corley, K. N., Olivier, A. K. and Meyerholz, D. K. 2013. Principles for valid histopathologic scoring in research. Vet. Pathol. 50, 1007–1015.

Hines, N. L. and Miller, C. L. 2012. Avian paramyxovirus serotype-1: A review of disease distribution, clinical symptoms, and laboratory diagnostics. Vet. Med. Int. 2012, 708216.

Hu, Z., Hu, J., Hu, S., Liu, X., Wang, X., Zhu, J. and Liu, X. 2012. Strong innate immune response and cell death in chicken splenocytes infected with genotype VIId Newcastle disease virus. Virol. J. 9, 208.

Khan, S. T., Rehmani, R. C. Miller, P. and Afonso, C. L. 2010. Phylogenetic and pathological characterization of Newcastle

disease virus isolates from Pakistan. J. Clin. Microbiol., 48: 1892–1894.

Li, R., Guo, K., Liu, C., Wang, J., Tan, D. Han, X., Tang, C., Zhang, Y.and Wang, J. 2016. Strong inflammatory responses and apoptosis in the oviducts of egg-laying hens caused by genotype VIId Newcastle disease virus. BMC Vet. Res. 12, 1–12.

Liu, J., Zhu, J., Xu, H., Li, J., Hu, Z., Hu, S., Wang, X., Liu, X. 2017. Effects of the HN antigenic difference between the vaccine strain and the challenge strain of Newcastle Disease virus on virus shedding and transmission. Viruses, 9, 225.

Miller, P. J. and Koch, G. 2013.Newcastle disease. Dis. Poult. 2013,13, 89–138.

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. 2009, 53, 39–49.

Miller, P. J., King, D. J., Afonso, C. L. and Suarez, D. L. 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine, 25: 7238–7246.

Moharam, I., Razik, A. A. E., Sultan, H., Ghezlan, M., Meseko, C., Franzke, K., Harder, T., Beer, M. 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 Pathol. 48, 406–415.

Nolan, T., Hands, R.E. and Bustin, S.A.2006. Quantification of mRNA using real-time RT-PCR. Nat. Protoc. 2006, 1, 1559.[CrossRef].

OIE, 2012. Newcastle disease. Chapter 2.3.14. OIE Manual of Standards for Diagnostic Tests and Vaccines, NB: Version adopted bythe World Assembly of Delegates of the OIEinMay 2012.

Palya, V., Kiss, I., Tatar-Kis, T., Mato, T., Felfoldi, B. and Gardin, Y. 2012. Advancement in vaccination against Newcastle disease: recombinant HVT NDV provides high clinical protection and reduction challenge virus shedding, with the absence of vaccine reaction. Avian Dis., 56: 282–287.

Radwan, M. M., Darwish, S. F., El-Sabagh, I. M., El-Sanousi, A. A. and Shalaby, M. A. 2013. Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes,47:311316.https://doi.org/10.1007/s11262-013-0950-y

Rehmani, S.F., Wajid, A., Bibi, T., Nazir, B., Mukhtar, N., Hussain, A., Lone, N.A., Yaqub, T. andAfonso, C.L. 2015.Presence of Virulent Newcastle Disease Virus in Vaccinated Chickens in Farms in Pakistan. J. Clin. Microbiol., 53, 1715–1718.

Sedeik, M. E., Elbestawy, A. R., El-Shall, N. A., Abd El-Hack, M. E., Saadeldin, I. M. and Swelum, A. A. 2019. Comparative efficacy of commercial inactivated Newcastle disease virus vaccines against Newcastle disease virus genotype VII in broiler chickens. Poult. Sci., 98 (5): 2000-2007.

Steel, R. G. D. and Torrie, J. H. 1960. Principles and Procedures of Statics; Mcgraw Hill Book Comp. Inc.: London, UK, 1960.

Sultan, H. A., Talaat, S., Elfeil, W. K., Selim, K., Kutkat, M. A., Amer, S. A. and Choi, K. S. 2020. Protective efficacy of the Newcastle disease virus genotype VII–matched vaccine in commercial layers.Poult. Sci. 2020, 99, 1275–1286.

Sultan, H. A., Arafa, A. E., Talaat, S., Gaballa, A. A., Kilany, W. H., Elfeil, W. K., Shehata, A. A. and Amarin, N. 2019. Efficacy of Clade 2.3.2 H5-Recombinant Baculovirus Vaccine in Protecting Muscovy and Pekin Ducks from Clade 2.3. 4.4 H5N8 Highly Pathogenic Avian Influenza Infection. Avian Dis. 2019, 63, 219–229.