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Research Article

Enhanced Protection and Blocked Viral Shedding for Broiler Chickens in Challenge with Newcastle Disease Virus Genotype VII by Generation of Oil Inactivated Vaccine Antigenically-Matched to The Endemic Virus in Egypt

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Abstract | The control of velogenic Newcastle disease virus (VNDV) is still a serious challenge, especially in endemic localities in Egypt. The phylogenetic proximity between used vaccines and field viruses can definitely protect against repeated NDV outbreaks. Therefore, the aim of the this work is to prepare a secure, sterile and potent an oil inactivated vaccine from a recent local isolate genotype VII 1.1 *"NDV-CH-EGY-GIZA-VVTNRC-2021"* and evaluates its efficacy against commercially available NDV genotype II vaccines in broiler chickens. Eighty chickens were housed in four groups (A, B, C and D) of 20 birds per group. Group A has been received the experimentally prepared inactivated genotype VII NDV vaccine by day 9 old, subsequently primed and boostered with commercial live attenuated genotype VII vaccine at 7 and 21 days-of age. While group B was treated with commercial live and killed genotype II NDV vaccines at the same days, respectively. Furthermore, groups C and D act as positive and negative non-vaccinated controls. At 30 days of age groups A, B and C were challenged with VNDV genotype VII1.1 isolate, where the clinical manifestations, gross post-mortem lesions, Humoral immune response and also quantification of virus shed post-challenge (PC) via real-time QRT-PCR were all screened and precisely recorded. The results revealed that, group A chickens developed the highest humoral antibody titers throughout the vaccination schedule and conferred a complete protection against mortality with milder clinical signs, moreover displayed a significant decrease in the shedding of NDV with drastically blocked shedding 7 days PC compared to group B with little clinical protection, higher mortalities, lower antibody titers and longest viral shedding PC. In conclusion, the NDV genotype VII-based vaccines homologous to challenge virus ensure a significant control on VNDV in terms of clinical protection, mortality, and virus shedding than the genotype II classic vaccines heterologous to the endemic virus in broiler chickens.

Keywords | Velogenic Newcastle disease virus, Experimentally prepared vaccine, Viral shedding quantification, Genotype VII-based vaccines, Broiler chickens

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INTRODUCTION

Newcastle disease Virus (NDV), caused by virulent class II avian paramyxovirus 1 (APMV-1), is a robustly infectious viral pathogen that struggles poultry production worldwide whilst strikes the poultry production with massive economic depletion due to elevated mortalities, declined egg production and extensive vaccination strategies to control it worldwide (OIE, 2012). NDV has an envelope with RNA genome encoding six proteins of about 15 kb; moreover, it's a single-stranded and negative-sense virus (Aldous and Alexander, 2001).

The high genetic diversity and the evolution of NDV are the main impacts of its continuous spread. So as to, NDV has been categorized into 21 genotypes (I–XXI) according to the recent characterization described by (Dimitrov et al., 2019). Genotype VII clade has been proven to responsible for frequent outbreaks in the poultry sector worldwide and particularly in Egypt since its first emergence in 2012 (Radwan et al., 2013). The sub-genotype VII 1.1 was found to be the most predominant circulated strain causing the recurrent outbreaks in Egypt several years ago according to (Moharam et al., 2019; Ahmed et al., 2022a; Amer et al., 2022), despite the wide-ranging vaccination protocols using both live and inactivated NDV vaccines applied in the poultry field.

The generality of commercial NDV vaccines are belonged to genotype I or II clades. Otherwise, NDV outbreaks caused by the field virus all over the world belongs to other genotypes, whereas the traditional genotype II used vaccines have been implemented for several decades ago to limit the extensive spread of the virus in the whole world (Kapczynski et al., 2013). Nevertheless, these vaccines never protect against the clinical disease or even stop the shedding of the antigenically-heterologous isolates particularly those belongs to the genotype VII clade (Choi et al., 2013; Roohani et al., 2015).

Efficient control of ND needs strict biosecurity rules and potent vaccination programs. Nevertheless, different factors interfere with obtaining a complete immunity post-vaccination under the field circumstances, in which genotype- incompatibility between the field virus and the vaccines strains probably the major reason for the inferior effectiveness of the commercially applied vaccines in the poultry farms (Ansori and Kharisma, 2020; Mahmud et al., 2022). The recent trend in VNDV control is how to generate vaccines comprising the endemic viruses, the so-named genotype-matched vaccines which provide not only a protective efficacy, but also stop the viral replication of the velogenic isolate post-infection (Bello et al., 2020), recently many authors developed genotype-matched vaccines either with lentogenic properties from recombinant NDV isolates (Cho et al., 2008; Roohani et al., 2015; Wang et al., 2020) or from a whole virus inactivated antigens (Ahmed et al., 2022b; Dewidar et al., 2022; Sultan et al., 2022), and they all proved the efficacy of these vaccines in controlling ND infection in the term of clinical protection, mortality and reducing the virus shedding after infection with genotype VII VNDV in chickens.

Accordingly, in the present study a sterile, safe, and efficient inactivated water in oil emulsified vaccine was prepared formulated in a modern efficient oil adjuvant. This vaccine is based on the currently endemic NDV isolate genotype VII 1.1 and evaluates its potency against conventional genotype II commercial vaccines in commercial broiler chickens.

MATERIALS AND METHODS

Challenge virus

The challenge virus used in this study was kindly supplied by the veterinary vaccines technology lab (VVT), National Research Centre, Egypt and characterized by sequencing as VNDV genotype VII1.1 designated as *"NDV-CH-EGY-GIZA-VVTNRC-2021"* with a Genbank accession number (MW603772). The virus was propagated via allantoic sac inoculation in ten-days-old specific pathogen-free embryonated chicken eggs (SPF-ECE) to obtain a challenge dose equal to 5.5 Log 10 embryo infective dose (EID50) per 0.5 ml via viral titration according to (Reed and Muench, 1938), which was administered intramuscularly to chickens (OIE, 2021).

Commercial vaccines

The experimental broiler vaccination program in this study includes three commercially available NDV vaccines; a live GII NDV vaccine (VOLVAC ND LaSota MLV ®) and an inactivated oil emulsion GII NDV vaccine (Volvac B.E.S.T AI ND®). Furthermore, alive genotype VII NDV attenuated vaccine (Himmvac® Dalguban N Plus), which were all supplied by a local agency.

Experimental local vaccine preparation

The inactivated experimental oil-emulsified ND vaccine was prepared from local NDV genotype VII1.1 isolate "NDV-CH-EGY-GIZA-VVTNRC-2021". The dilution of virus was occurred via 10-fold dilutions in sterile phosphate buffer saline and 100 µl of the virus suspension that was inoculated in the allantoic cavity of 11days-old ECE and then incubation was carried out for 5 days at 37°C. The allantoic fluid was then aspirated and tested for haemagglutination (HA) activity. Furthermore, it was examined to be sterile from Mycoplasma, bacteria or fungi contamination. The NDV vaccine seed strain was adjusted to 8.2 Log 10 EID50 as a final virus titer

following to procedures of Reed and Muench (1938). Moreover, the allantoic fluid harvest was then inactivated by addition of ultra-purified formaldehyde solution 37 % at a final assembly of 0.003% with a thoroughly stirring continued for 24 hours at room temperature (OIE, 2021). Two blind passages in 11 days-old ECE were carried out to the inactivated antigen samples for the achievement of complete inactivation. Moreover, the HA activity occurrence was tested after every passage because NDV is seems to be fully inactivated if there is no embryo deaths or HA positive results. Finally, mixing was occurred to the inactivated antigen as water in oil emulsified vaccine by blending with the SEPPIC Montanide™ ISA71® mineral oil at 30/70% ratio according to the standard protocol of manufacturer instructions.

The sterility test was carried out to ensure that the ND vaccine was clear from any contamination of bacteria or fungi via cultivation of the vaccine samples on tryptose soya agar media and Saburaoud dextrose agar. On the same side, a safety test was achieved on a group of 20 chicks 4 weeks-old via injection of the prepared vaccine in a double dose of 1 ml subcutaneously and monitoring till 7 days post-inoculation for any symptoms of local adverse unfavorable reactions or any NDV clinical signs.

Serology

Blood serum was collected every week randomly from 10 birds per group pre and post-challenge. The haemagglutination inhibition (HI) test was applied to estimate NDV antibody titers using twofold serum dilutions and 1% chicken erythrocytes with 4 HA units of LaSota virus according to the procedures of (OIE, 2021).

Virus shedding

Virus shedding was tested by quantitative real-time (QRRT-PCR) from both oropharyngeal and cloacal swabs at 3, 5 and 7 days pc. NDV-specific primer and probe were used to amplify the VNDV fusion protein gene and to differentiate between the velogenic challenge isolate and the lentogenic vaccinal strains according to Al Habeeb et al. (2013) following their procedures in thermal cycling conditions.

F+ 4839 5'-TCCGGAGGATACAAGGGTCT-3'
F- 4939 5'-AGCTGTTGCAACCCCAAG-3'
Fusion F- 4894 (probe) 5'-[FAM] AAGCGTTTCT-GTCTCCTTCCTCCA [BHQ-1]-3'

The virus titer of each sample was determined relative to a standard curve of ten-fold dilutions of the total viral RNA copies of the challenge NDV strain, in which this standard curve allowed the estimation of unknown virus titers based on their threshold cycle (Ct) values. Results were expressed as the titer of challenge virus per ml of swabs (Log-10) as each sample of 2 $\log_{10}$ or more was considered positive (cut-off value is 2 $\log_{10}$).

Experimental vaccination and challenge design

Eighty-one-days old commercial broiler chicks (Ross 308®) were divided into 4 groups of 20 birds to evaluate the efficacy of experimentally prepared and commercial NDV genotype VII and II vaccines. Whereas, group A received commercial live attenuated NDV vaccine genotype VII at 7 and 21 days old intraocularly (eye drop) and locally prepared inactivated NDV vaccine genotype VII at 9 days of age subcutaneously (S/C). While group B was treated with both commercial live attenuated and inactivated NDV genotype II LaSota strain with the same routes at 7, 21 and 9 days old, respectively also. Furthermore, group C served as a non-vaccinated challenged positive control. Finally, group D was neither vaccinated nor challenged negative control. At 30 days of age groups A, B, and C were challenged with NDV genotype VII1.1 isolate *"NDV-CH-EGY-GIZA-VVTNRC-2021"* with a challenge dose equal to 5.5 Log_{10} (EID_{50}) per 0.5 ml, which was administered intramuscularly (I/M) to chickens (Table 1).

Table 1: Experimental design of vaccination and challenging trial in broiler chickens (n=20 birds in each group).

Groups	Vaccination regime			Challenge at 30 days old [5]
	NDV vaccine	Age/days	Volume and method	
A	Live commercial NDV. GVII[1] W/O Exp.Inact.NDV GVII [2]	7 & 21 9	50 µl eye drop 0.5 ml (S/C)	0.5 ml (I/M)
B	Live commercial NDV.GII [3] Inact commercial.NDV GII [4]	7 & 21 9	50 µl eye drop 0.5 ml (S/C)	0.5 ml (I/M)
C	None	None	None	0.5 ml (I/M)
D	None	None	None	None

[1]Live attenuated commercial NDV genotype VII vaccine. The vaccinal dose equals 6-$\log_{10}$ EID_{50} / bird given via eye drop route. [2]Inactivated water in oil emulsion experimental locally prepared NDV vaccine genotype VII. The vaccinal dose equals 8.2-Log_{10} EID_{50} given 0.5 ml/bird via the subcutaneous route (S/C). [3]Live attenuated NDV genotype II vaccine. The vaccinal dose equals 6-$\log_{10}$ EID_{50} / bird given via eye drop route. [4]Inactivated oil emulsion commercial NDV genotype II vaccine. The vaccinal dose equals 8.2-Log_{10} EID_{50} given 0.5 ml/bird via the subcutaneous route (S/C). [5]Challenge with velogenic Newcastle disease virus (genotype VII1.1). The virus challenge dose equals 5.5-Log_{10} EID_{50} given 0.5 ml/bird via the intramuscular route (I/M).

Statistical analysis

To analyze data and detect the significance of differences and standard deviation (SD) among vaccinated groups and corresponding controls the SPSS 21 software was used via One-way ANOVA. The significance was assured when a probability (p) value ≤ 0.05.

RESULTS AND DISCUSSION

Stability tests for experimental prepared vaccine

Our results declared that, the enhanced water in oil prepared vaccine formed an emulsion that was still unflawed and intact for more than 3 months at 20 °C and 8 months at 4 °C. This pointed to the stability and validity of the prepared vaccine emulsified in an oil phase without any separation of its contents.

Sterility and safety tests for experimental prepared vaccine

The tested experimental ND vaccine samples on the cultured media revealed a sterile vaccine with no existence of any bacterial or fungal contamination. On the same side, the experimental ND vaccine safety investigation declared that, the inoculated birds didn't display any regional reactions or distressing clinical manifestations during the screening period.

Clinical signs and morbidity

The clinical signs and morbidity of this study were monitored daily for 7 days pc. Where, group A shows little to mild clinical signs of slight depression, decrease in food intake, little greenish diarrhea in a few birds and mild respiratory manifestations with no recorded nervous signs. While in group B displayed moderate signs of marked depression with an obvious decrease in food intake, respiratory signs and rales, greenish diarrhea and appearance of mild nervous signs of recumbency and twisted wings. On the other hand, the non-vaccinated challenged control group C recorded very severe signs including; off-food, severe depression, acute respiratory manifestations with obvious nasal discharges, greenish diarrhea in all birds, and pronounced nervous signs of torticollis and dropped wings with complete recumbency ended with death. Finally, the non-vaccinated non-infected group D has no recorded clinical adverse reactions or even morbidity till the end of the experiment (Table 2).

Post-mortem gross lesions (Pm)

In necropsy examination, the pm lesions were recorded as; petechial hemorrhagic red spots were observed in the proventriculus with ulcerated cecal tonsils and splenomegaly as well as, severe tracheitis was recorded in non-vaccinated infected control group C (Figure 1). Meanwhile, mild or even no Pm lesions were monitored in both immunized-infected groups A and B with no recorded lesions at all in negative control group D.

Table 2: Scoring of clinical manifestations in challenged chickens regarding the severity pc.

Group	Recorded clinical signs				
	Depression	Food intake	Greenish diarrhea	Respiratory signs	Nervous signs
A	Mild	Mild	Mild	Mild	None
B	Moderate	Moderate	Moderate	Moderate	Mild
C	Severe	Off- food	Severe	Severe	Severe
D	None	None	None	None	None

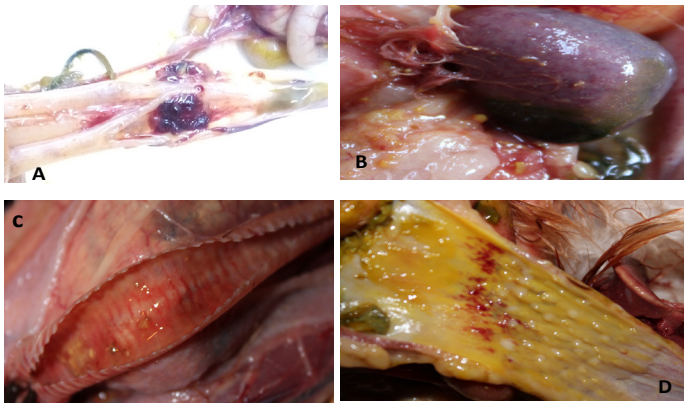

Figure 1: Post mortem gross lesions recorded in control group C 3 days pc. A: ulceration and hemorrhages in caecal tonsils. B: Marked splenomegaly. C: severe bloody tracheitis. D: hemorrhagic spots and petechial hemorrhages on proventriculus.

Mortality and protective efficacy of the vaccination-challenge trial

The mortality rate and protection level were assessed after challenge with VNDV genotype VII strain *"NDV-CH-EGY-GIZA-VVTNRC-2021"* at 30 days-old and recorded as; 0 % mortality (n= 0/20) in group A of locally prepared vaccine with protection rate 100%, and group B treated with genotype II NDV vaccines showed 25% mortality (n=5/20) with 75% subsequent protection rate declaring significant protection from mortality towards group A compared with group B. While in control group C all birds died by the day 5 pc with 100% mortality (n=20/20). Meanwhile, control group D has no recorded mortalities at all (Table 3).

Assessment of humoral immune response

The humoral immune response to NDV-tested vaccines was assessed in commercial broiler chickens vaccinated with either NDV genotype VII vaccines or NDV genotype II commercial vaccines. The results (Table 4, Figure 2) showed that the NDV antibody titers of both groups A and B were increased gradually throughout the experiment and reached their peaks about 3 weeks post-primary

Table 3: Mortality and protection % in vaccinated and challenged birds post-challenge.

Group/ no of birds	Vaccination schedule	Daily monitoring of birds post-challenge							Total no. of dead birds	Protection %
		1	2	3	4	5	6	7		
A (20)	Live. GVII Inact. NDV GVII	None	None	None	None	None	None	None	0	▶100
B (20)	Live.GII Inact.NDV GII	1	2	1	1	None	None	None	5	75
C (20)	None	2	3	3	5	7	None	None	20	0
D (20)	None	None	None	None	None	None	None	None	0	NT

NT: none tested; None: not treated; ▶: denotes significance from groups B and C (P<0.05).

Table 4: Humoral antibody responses of vaccinated chickens challenged with VNDV *"NDV-CH-EGY-GIZA-VVTNRC-2021"* at 30 days old.

Group no./ Birds no.	Vaccination regime		HI titer means SD Log-2 at age/days (N = 5)				
	Type	Age/days	7	14	21	28	35
A (20)	Live. GVII Inact.NDV GVII	7&21 9	5.2.00±1.41	6.00±0.83	▶7.5±0.83	▶8.3±0.83	9.5±0.83
B (20)	Live.GII Inact.NDV GII	7&21 9	5.4±0.89	5.8 ±0.83	6.4 ±1.14	7.00±0.83	8.3±0.89
C (20)	Non vaccinated control	None	4.1±1.00	3.5±0.54	2.00±0.70	0.6±0.54	NT
D (20)	Non vaccinated control	None	4.00±1.00	3.4±0.54	2.1±0.70	0.7±0.54	0.20±0.54

Nt: none tested; None: not treated; ▶: denotes significance from groups B, C, and D (P<0.05).

Table 5: Viral shedding after vaccination trial and challenge with VNDV genotype VII 1.1 "NDV-CH-EGY-GIZA-VVTNRC-2021" at 30- days of age in broiler chickens.

Group no.	Birds no.	Vaccination protocol		Shedding at days post challenge *					
		Type	Age / days	Oropharyngeal swabs			Cloacal swabs		
				3	5	7	3	5	7
A	20	Live. GVII Inact.NDV GVII	7 & 21 9	5/10	2/10	▶0/10	2/10	4/10	▶0/10
B	20	Live.GII Inact.NDV GII	7 & 21 9	7/10	5/10	2/10	4/10	7/10	3/10
C	20	None	None	10/10	■5/5	NT	10/10	■5/5	NT
D	20	None	None	0/10	0/10	0/10	0/10	0/10	0/10

*Swabs were randomly taken from ten birds in each group and evaluated to quantify virus shedding via real-time RT-PCR. The frequency of birds detected with challenge virus is expressed as the number of positive swabs/total number of swabs tested. ▶: denotes significance from groups B, C, and D at (P<0.05). ■: 5 birds only remained at the tested day. NT: not tested. None: not treated.

immunization and even though post-challenge with significantly higher titers from control groups C and D. On the other hand, the antibody curve of non-immunized control groups C and D was gradually declined afterward. Furthermore, groups A and B were analogized to each other in which group A exhibited a higher significant antibody level compared to group B especially at 2 and 3 weeks post primary vaccination with HI titers 7.5 $\log_2$, 8.3 $\log_2$ and 6.4 $\log_2$, 7 $\log_2$, respectively. The HI titers were still detectable in high levels post-infection in challenged groups A and B at 9.5 and 8.3 $\log_2$, respectively one week pc.

Virus shedding post-challenge

Viral shedding load is an important aspect that can reflect the potency of the vaccine as oral and cloacal shedding is the primary source of viral transmission among the chicken flocks. Oropharyngeal and cloacal swabs were collected at 3, 5, and 7 days pc randomly taken from ten birds in each group and quantified via real-time Q_rRT-PCR then expressed as the number of positive swabs/ total number of tested swabs. Results declared that all swabs collected from control group C were positive for NDV at the cut-off value (2 $\log_{10}$) and continued for at least 5 days pc. While virus shedding from group A was significantly with lower load at 3 and 5 days pc in compared to group B and shedding was blocked completely at 7 days pc in group A in all tested swabs, meanwhile, group B continue to shed virus at least 7 days pc from both oral and cloacal routes. Therefore, the experimentally prepared genotype VII NDV vaccine provided the shortest and lower viral load among all challenged groups A, B, and C, while commercial genotype II vaccines showed prolonged and higher shedding results (Table 5, Figures 3, 4).

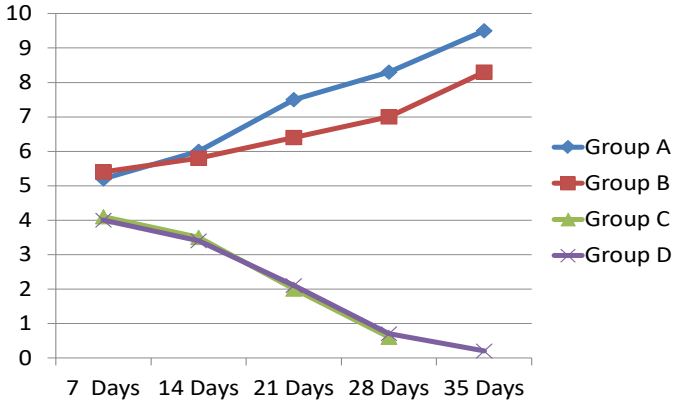

Figure 2: Results of humoral antibody curve throughout the vaccination course and after challenge with VNDV *"NDV-CH-EGY-GIZA-VVTNRC-2021"* in all experimental groups A, B, C, and D.

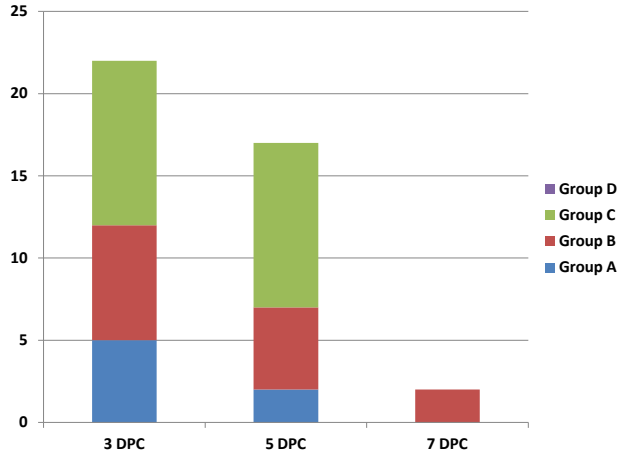

Figure 3: Oropharyngeal viral shedding at days post-challenge (DPC) in all tested groups shows a decrease and stop of shedding in vaccinated group A with NDV GVII vaccines compared to other tested groups.

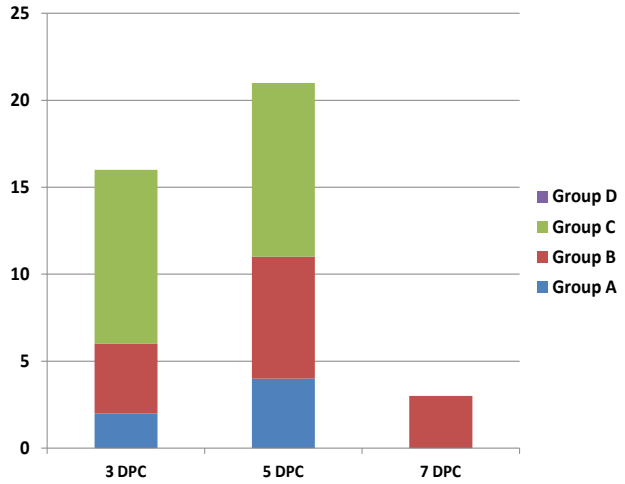

Figure 4: cloacal viral shedding at days post-challenge (DPC) in all tested groups shows a decrease and stop of shedding in vaccinated group A with NDV GVII vaccines compared to other tested groups.

Despite the implementation of ND vaccines several decades ago, high economic losses from frequent ND outbreaks still occurs annually, demonstrating that the antigenic variation between field virus and vaccinal strains is the major cause of vaccination failure especially genotype VII outbreaks all over the world, as non-genotypically matched vaccines currently used may not provide the sufficient protection from ND challenge (Amer et al., 2019; Ahmed et al., 2022b) In the current study, the efficacy of experimentally prepared killed genotype VII ND vaccine from recent local isolate *"NDV-CH-EGY-GIZA-VVTNRC-2021"* was evaluated against commercial ND vaccine genotype II, once the prepared vaccine was approved to be safe and sterile with validity to apply in commercial broiler chickens, and the establishment of quality control operations for the prepared vaccine showed its sterility with no bacterial or fungal contaminants. Also, no systemic or adverse local reactions and mortalities were recorded in treated chicks, and these guarantees the safety of the prepared antigen comes in consent with OIE standards of vaccines operations (OIE, 2021).

To test the practical immunogenicity and protective efficacy of both experimentally prepared and commercially inactivated NDV vaccines from a single shoot only, they were primed and boostered by live attenuated NDV vaccines of the same genotype to each vaccinated group. In this experiment, to avoid the conceivable maternal antibodies neutralization (which decreased by half every 6-7 days) to the applied vaccines, live NDV vaccine was primarily employed at 7 days old. Moreover, booster live ND vaccines were applied to achieve the secondary immune reaction (Vrdoljak et al., 2018). While Miller et al. (2007) concluded that, inactivated vaccines are less influenced by maternal antibodies and produce higher solid immunity and neutralization titers.

The results of this study reflect the protection levels obtained pc, where NDV genotype VII vaccines provided significant protection against mortality (100% protection, 0% deaths) and also against clinical disease compared with genotype II vaccines (75% protection, 25% deaths). Whereas, non-vaccinated challenged group C showed 100% mortality with severe and prominent clinical signs of respiratory, digestive, and nervous manifestations. The weak clinical signs found in group A compared to other challenged groups may be likely to the specific humoral antibody provided by identical ND vaccines to the challenge virus, thus increasing the close harmony between the challenge virus and the vaccine seed that enhance the clinical protection against developing serious clinical signs as well as protection levels (Liu et al., 2017). These results come in concordant with (Adi et al., 2019; Amer et al., 2019; Aljumaili et al., 2020; Dewidar et al., 2022; Sultan et al., 2022) who stated that the NDV vaccine seed

closely related to genotype VII virus conferred a higher protection against mortality with minimal morbidity and clinical disease. Also, similar results to our study concluded that, no obvious clinical signs with little mortalities were observed in birds receiving killed genotype VII NDV vaccines after a challenge with VNDV genotype VII (ZJ1 strain) as formerly mentioned by (Hu et al., 2009). On the other hand, Sedeik et al. (2018) concluded that the use of homologous ND-prepared vaccines did not induce sufficient protection against clinical disease or even deaths because of obvious nervous and respiratory manifestations in all vaccinated challenged birds. Also, Dortmans et al. (2012) and Kapczynski et al. (2013) declared that adequate protection against mortality and clinical illness from the VNDV challenge could be obtained when immunization of birds with live and/ or inactivated LaSota strain NDV vaccines.

Since the clinical signs and mortality protection rate are important in potency assessment, gross pm changes are also valuable, which commonly appeared as; tracheal, spleen, proventricular and cecal tonsils hemorrhages with enlarged and mottled or even necrotic spleen that are typically seen in our experiment and previously referenced by Susta et al. (2011); Miller and Koch (2013) that are mainly recorded in susceptible infected non-vaccinated controls.

Based on the humoral serological findings in our study, it needs to stress that the better valuing of protection in birds against NDV challenge is achieved with the successive level of protective antibodies. Furthermore, the pertinence between antibody titers and the level of protection to NDV infection is usually more productive in birds immunized with killed vaccines as the humoral antibodies is the major immunological response to inactivated antigen (Vrdoljak et al., 2018). In the current study, the humoral antibody titers were screened by HI test in chickens immunized with live and killed genotype VII and II NDV vaccines. The results showed that the significantly higher antibody titers of 7.5 and 8.3 $\log_2$ at 21 and 28 days-old, respectively were recorded in group A immunized with NDV genotype VII vaccines in compared to Group B of genotype II treated vaccines. However, the non-vaccinated group C and D showed a gradual decrease in titers till the challenge day at 30 days-old. Therefore, the experimentally prepared NDV vaccine induce the highest protective HI titers and enabled the challenged birds to exhibit better clinical protection and optimum efficacy against mortality. These results are harmonized with the recently reported by (Cheng et al., 2016; Mahmoud et al., 2019; Bello et al., 2020). Conversely, Adi et al. (2019) found lower antibody titers induced by genotype VII NDV vaccines than of commercial genotype II, although it could fully protect chickens. In addition to, Sultan et al. (2022) findings also indicated a non-significant difference in antibody titers in the different immunized groups, whatever the genotype of the ND vaccine applied.

The control and limitation of NDV spread among chicken flocks are mainly correlated with the amount of viral load. Therefore, the assessment and quantification of viral shedding is one of the major aspects in the judgment of vaccine efficacy. In the current study, virus shedding was estimated by QRRT-PCR via aggregation of oral and cloacal swabs at days 3, 5 and 7 pc. The results declared that group A immunized with the experimental NDV genotype VII vaccine significantly showed the most reduced viral shedding load in all tested days comparing to other vaccinated and challenged groups B and C. Furthermore, the NDV genotype VII vaccine was capable of blocking viral shedding completely one week pc in both oropharyngeal and cloacal swabs achieving the little and shortest amount of viral shedding results in terms of the number of negative tested swabs and percentage of the shedding virus among all challenged groups in this experiment. Similarly, and in parallel with our results (Liu et al., 2017; Amer et al., 2019; Bello et al., 2020; Wang et al., 2020) mentioned that the birds that had been immunized with genotype VII vaccines homologous to the challenge virus presented a prominent or even significant reduction in viral shedding at days or weeks post-challenge. In addition, Dewidar et al. (2022); Sultan et al. (2022) concluded that, the inactivated ND vaccines antigenically-matched to field virus could upgrade the control approaches for VNDV in chicken flocks due to the effect of locally prepared vaccines formulations closely related to the epidemic virus, which provide high titers of more specific antibody than the traditional genotype II NDV vaccines, which proved not efficient to overcome the repeated NDV genotype VII challenges.

CONCLUSIONS AND RECOMMENDATIONS

The introduction of genotype VII-matched NDV vaccines either commercially available or autogenously prepared leads to the induction of a highly specific antibody response, as well as provides better clinical protection and enhances the optimum protection from mortality. Moreover, found promising in controlling NDV spread by reducing or even blocking viral shedding against challenge with currently circulated and endemic NDV genotype VII 1.1 in broiler chickens.

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NOVELTY STATEMENT

In our study, an inactivated emulsified NDV genotype VII vaccine was developed from a predominant local isolate and evaluated against commercial NDV genotype II vaccines and proved to be a promising vaccine trial to overcome NDV spread and control the recurrent outbreaks in commercial broiler chickens.

AUTHOR'S CONTRIBUTION

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

Ethical approval

The chicken experiments were carried out following the standard procedures of animal care and handling and under approval of the Medical Research Ethical Committee (MREC) at the National Research Centre, Dokki, Cairo, Egypt with registration no; 3337082022.

Consent to publish

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Conflict of interest

The authors have declared no conflict of interest.

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