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Research article

Comparative analysis of commercial recombinant vaccination strategies against Newcastle disease in broilers

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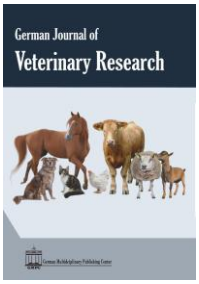

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Abstract

The widespread Newcastle disease (ND) genotype VII among vaccinated and unvaccinated chicken populations makes it one of the most threatening viruses impacting poultry, causing significant losses in the poultry industry. Recently, recombinant vector vaccine technology has proven to induce a durable protective immune response, even in the presence of maternal antibodies, thereby addressing the limitations associated with traditional vaccines. This study evaluated the protective efficacy of the recombinant turkey herpesvirus double construct vaccine (HVP360) that encodes both the NDV-F and IBDV VP2 genes alongside the recombinant fowl pox-based NDV vaccine (vFP96.5) and their combined use against virulent NDV genotype VII. For this purpose, 125 one-day-old broiler chicks (Cobb) were divided into five groups (G1-G5), in which chickens kept in G1 were unvaccinated unchallenged. Chickens kept in G2, G3, and G4 were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively. At 28 days of age, half of the birds (n=15) in each vaccinated group (G2, G3, and G4) and the G5 (positive control group) were challenged intraocularly with 10^6 EID_{50} velogenic NDV genotype VII. Protection was assessed based on mortalities, clinical signs, seroconversion, and viral shedding. Results demonstrated significant protection offered by the recombinant vaccines, reaching a remarkable level of 93.33% for G2 and G4, followed by 71.42% for G3, associated with a notable reduction of clinical signs and lesions. In contrast, the mortality rate reached 75% in unvaccinated challenged G5. Significant differences in seroconversion based on the hemagglutination (HI) test and ELISA were observed among vaccinated groups on days 14, 21, and 35, in which chickens kept in G3 exhibited the highest antibody titers, followed by G4 and G2. Viral shedding was significantly reduced in all vaccination groups at 3, 5, and 7 days post-challenge, with the highest reduction in dual-vaccinated chickens (G4) followed by G2. In conclusion, the concurrent application of HVP360 and vFP96.5 in combating the ND virus can establish a foundational basis for the vaccination program aimed at one-day-old chicks.

Keywords: Newcastle disease, Recombinant vaccine, HVP 360, FP96.5

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Introduction

Newcastle disease (ND) poses a significant challenge worldwide, particularly in endemic regions, where it is linked to extremely high mortality rates that can reach nearly 100%. It also negatively impacts weight gain and feed efficiency and decreases egg production, fertility, and hatchability (Hines and Miller, 2012; Miller and Koch, 2013; Suarez et al., 2020). The ND virus, known as avian paramyxovirus-1, belongs to the genus Avulavirus within the *Paramyxoviridae* family (Hines and Miller, 2012). It is an enveloped, single-stranded, negative-sense, non-segmented RNA virus encoding a minimum of six structural proteins: hemagglutinin-neuraminidase (HN), fusion protein (F), nucleoprotein (NP), phosphoprotein (P), matrix protein (M), and large RNA polymerase (L). Based on the clinical and pathological observations in infected avian species, the virus can be categorized into five distinct pathotypes:

lentogenic, mesogenic, viscerotropic velogenic, neurotropic velogenic strains, and asymptomatic enteric (Czeglédi et al., 2006; Suarez et al., 2020). Genetically, Newcastle disease virus (NDV) is classified into two main categories: class I and class II viruses. Class I NDV, primarily found in wild avian species, exhibits low virulence and is infrequently observed in poultry populations (Miller and Koch, 2013; Snoeck et al., 2013). Based on the nucleotide sequence analysis of the F gene, class II viruses are further categorized into 18 distinct genotypes (I to XVIII), with genotypes V, VII, and VIII being the most prevalent globally (Sultan et al., 2021).

Even with extensive vaccination efforts utilizing both live and inactivated ND vaccines across different protocols, clade VII.1.1 of sub-genotype VII.1 from class II NDV remains the dominant strain responsible for the fourth ND pandemic, which persists to this day, impacting regions in Asia, Africa, Europe, and South America (Rui et al., 2010; Dimitrov et al., 2016; Suarez et al., 2020; Sultan et al., 2021). In 2011, the Giza Governorate reported the initial identification of genotype VII, with sub-genotype VII.1.1 (VIId) emerging as the most widespread strain in Egypt, leading to numerous outbreaks of NDV in poultry (Radwan et al., 2013). The primary clinical manifestations of ND in broilers included significant lethargy, greenish diarrhea, paralysis, and a noticeable mortality rate occurring within 48 to 72 hours after the onset of initial symptoms. Additionally, affected birds exhibited severe conjunctivitis, facial swelling, drooping wings, and general dullness. Necropsy findings revealed meningeal and subcutaneous congestion along with congestion in the liver, spleen, and lungs. There was also an enlargement of the gallbladder, accompanied by tracheitis and air sacculitis. Hemorrhagic lesions were observed at the margins of the proventriculus glands, along with ulcers in the gastrointestinal tract and cecal tonsils (Abdel-Moneim et al., 2006; Ewies et al., 2017). The virus can be transmitted via direct contact with feces and respiratory secretions, as it is released during the incubation phase and for a brief time during recovery (Alexander and Senne, 2008; Alexander, 2009).

The lentogenic strains of NDV that are most utilized as seed strains for both live and inactivated ND vaccines include LaSota, Hitchner B1, Ulster, and VG/GA (Dimitrov et al., 2017a; Dimitrov et al., 2017b). Live vaccination has several drawbacks, notably the interference with antibodies acquired from the mother, the possibility of respiratory reactions post-vaccination, and the risk of virulence reversion. This reversion may elevate the chances of subsequent bacterial infections, leading to production losses and the potential for horizontal transmission (Winterfield et al., 1980).

Recently developed techniques, known as viral vector vaccines, use innovative molecular biological procedures to carry the genetic code of the target pathogen's protective genes through a vector virus. A novel recombinant vaccine utilizing a genetically modified turkey herpes virus has been introduced to overcome the limitations associated with traditional vaccination. This vaccine is based on the herpesvirus of turkeys (HVT) from the FC126 strain, into which the F gene responsible for the immunogenicity of NDV has been integrated. This advanced approach protects against Marek's disease virus and virulent NDV (vNDV) (Morgan et al., 1992; Morgan et al., 1993; van Hulten et al., 2021). Furthermore, a fowlpox-based recombinant virus (vFP96.5) has been developed and is now commercially available. It expresses F and HN glycoprotein derived from a virulent NDV strain. In commercial chickens that received this vaccination, substantial protection was observed following NDV exposure (Taylor et al., 1996; Romanutti et al., 2020; Wang et al., 2024).

This study aimed to evaluate the efficacy of different recombinant vaccines against NDV, with a particular focus on recombinant HVT and fowlpox vaccines and their combinations, without considering any immunological assistance from other ND vaccines.

Material and methods

Ethical approval

The Scientific Research Ethics Committee of the Faculty of Veterinary Medicine at Suez Canal University in Ismailia, Egypt, has approved the protocol and materials utilized in this scientific study under approval number 2022039.

Recombinant vaccines

In this study, two commercially available recombinant ND vaccines were utilized in the vaccination protocols for broiler chicks. The first is the HVT-double-vector vaccine (International Free Trade, Cairo, Egypt), referred to as the

HVP360 strain. This vaccine encodes both NDV-F and IBDV VP2 genes; the F gene was inserted with an upstream immediate-early (IE1) promoter derived from the human cytomegalovirus (HCMV) and a downstream CMV terminator. The second vaccine is recombinant vFP96.5 (International Free Trade, Cairo, Egypt), developed by inserting the F and HN cDNAs into the non-essential open reading frame of the genome. This specific open reading frame, designated as F8 in this laboratory, is located at the junction of the 2.0 kbp and 14.2 kbp HindIII fragments, approximately 94 kbp from the right end of the FPV genome. Both vaccines were processed and prepared following the manufacturer's instructions and were administered subcutaneously at a dosage of 0.2 mL per bird to one-day-old chicks.

Challenge virus

The challenge virus utilized in this study was a Velogenic NDV (accession number: MZ409479), obtained from the reference laboratory for quality control on poultry production at the Animal Health Research Institute (AHRI), Giza, Egypt. Sequencing analysis confirmed its classification as vNDV genotype VII (Data not shown).

Experimental birds and experimental design

A total of 125 one-day-old broiler chicks (Cobb) were obtained from a commercial hatchery located in Cairo and evaluated for maternally derived antibodies (MDA) against the NDV through hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) methods. The chicks were housed in completely isolated experimental rooms, thoroughly cleaned, and disinfected under natural daylight exposure. They were provided with unrestricted access to commercial chicken feed and water. The chickens were divided into five groups, designated as G1, G2, G3, G4, and G5 (n=25 chicks/group). They go through various vaccination protocols, as illustrated in Figure 1. At day 28 of age, the vaccinated groups from G2 to G4 were subdivided into challenged and unchallenged subgroups ($G2^-$, $G3^-$ and $G4^-$) to evaluate the performance and the specific humoral immune response of the vaccines until the end of the experiment at day 42. Birds were challenged with 10^6 EID_{50} of widely circulating vNDV genotype VII intraocularly.

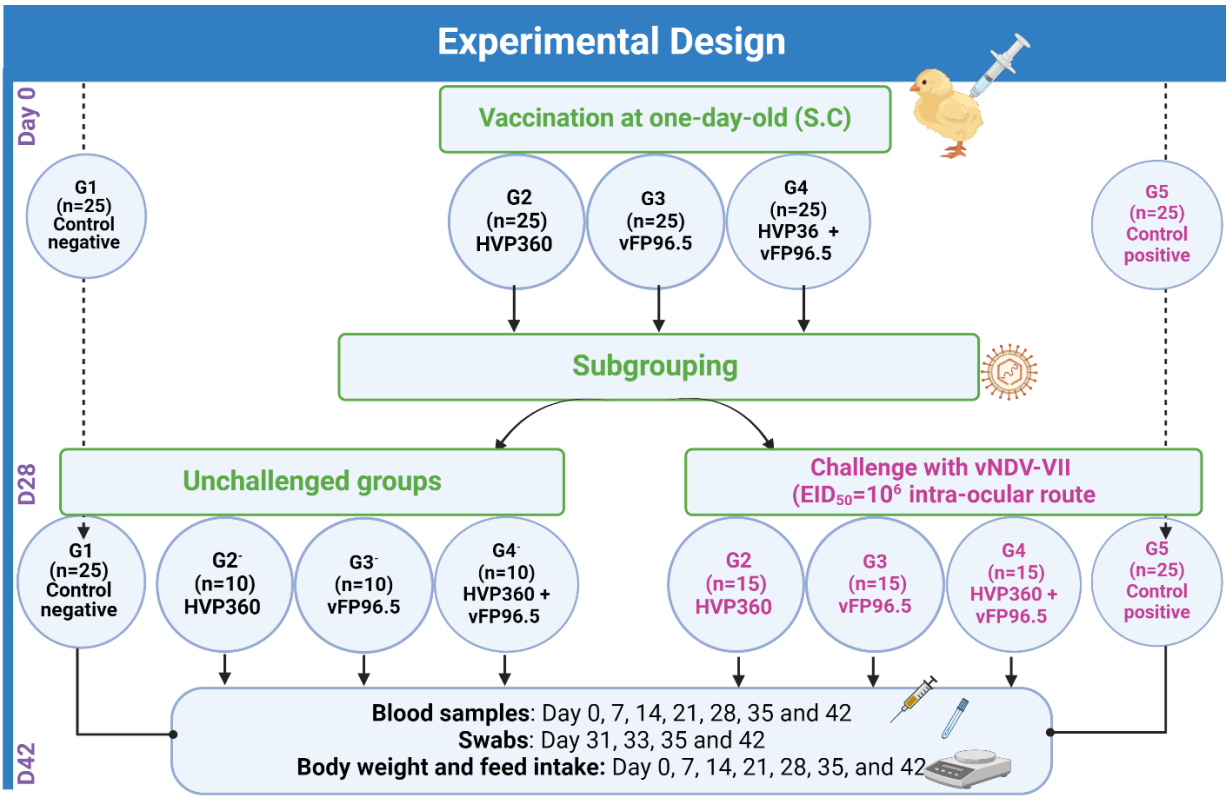

Figure 1: Design of experimental protocols for broiler (Cobb) vaccination utilizing different recombinant NDV vaccines.

Assessment parameters

Body performance

Body performance was evaluated weekly starting from day one of life by calculating the feed intake and individual weight gain in each group then, taking the means, and comparing them with negative control G1 and positive control G5 before and after the virus challenge to identify distinctions and quantify the differences (Amer and El-Ghany, 2006; Ellakany et al., 2019).

Humoral antibody response to NDV vaccines

Five blood samples were collected per group on days 0, 7, 14, 21, 28, 35, and 42. The chicken blood was drawn from the wing vein, maintained at 37°C for one hour, and refrigerated at 4°C overnight. The serum was separated through centrifugation at 2000 rpm for 10 minutes and stored at -20°C until assayed. The HI test was performed using NDV Genotype II (Guangdong Wenshi Dahuanong Biotechnology Co., Ltd. Guangdong, China) as the antigen, prepared at 4 HA units in a V-shape 96-well microplate following the guidelines outlined in the OIE manual (OIE, 2015). Additionally, anti-NDV antibodies were identified using ELISA kits based on F antigen (Innovative Diagnostic ID—0416 GB, batch number L43, Pharmachime, Cairo, Egypt), according to the manufacturer's instructions, with a positive sample having a value exceeding 993.

Clinicopathological observation, survival rates after virus challenge

Over the 14 days following the challenge, chickens were closely monitored for typical clinical signs of virulent NDV-VII, mortalities, and survival rates to determine the level of protection. Dead birds were necropsied, and pathological changes were recorded.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for detection of NDV shedding post-challenge

On the 3rd, 5th, 7th, and 14th days after the challenge, three tracheal and cloacal swabs were randomly collected from each group and suspended in sterile phosphate-buffered saline. The tracheal swabs were processed separately, while the cloacal swabs from each group were pooled together for each collection. RNA extraction was performed using the QIAamp RNeasy Mini Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). The qRT-PCR was conducted using the GoTaq® 1- step RT-qPCR kit (Promega®, USA, Madison, USA). The reactions were carried out in a StepOne real-time PCR system, employing the specified primer pairs and probe target the M-gene of vNDV-VII, according to Wise et al. (2004).

Statistical analysis

A one-way analysis of variance (SAS, 2012) was used to analyze the data, followed by a post hoc Duncan test using SPSS. Results considered significant at p-value <0.05. Data were denoted as mean ± standard error of the mean, with viral counts expressed in Log_{10} format. The Levene and Shapiro–Wilk tests were conducted to check for normality and homogeneity of variance (Razali and Wah, 2011). The variation within groups was examined through the analysis of variance (SAS, 2012). Figures were fitted by the GraphPad Prism software (Graph Pad version 5.0, USA).

Results

Clinical symptoms, PM lesions, and mortalities after NDV-VII challenge

Symptoms observed beginning on the 4th day post-challenge (pch) included signs of depression, a slight decrease in both feed and water consumption, and conjunctivitis accompanied by ocular discharge. Additionally, there were rales, greenish diarrhea, a recumbent position, and neurological manifestations (Figure 2: a-d). These symptoms were prominently seen in the positive control challenged G5, followed by groups G3, G2, and G4 according to severity, whereas no symptoms were noted in the non-vaccinated-non-challenged G1 (Table 1). The postmortem findings ranged from mild to severe, with the non-vaccinated challenged G5 exhibiting the most pronounced lesions. These included congested pectoral muscles, congestion and exudate in the tracheal mucosa, a mottled spleen, swollen kidneys, ulcers in the small intestine, and hemorrhaging at the tips of the proventriculus glands and ileocecal tonsils. In contrast, the severity of lesions was less pronounced in the other vaccinated groups (Figure 2: e-g). Mortality data collected for 14 dpc indicated a highly significant impact of vaccination on the survival rates of the challenged groups (p<0.0001). The unvaccinated unchallenged G1 achieved the highest survival rate at 100%, followed by groups G2 and G4 (93.33% each).

Conversely, group G3 demonstrated a lower survival rate of 71.42% despite vaccination. Notably, the unvaccinated-challenged G5 experienced elevated morbidity and mortality, with only 25% of the birds surviving (Figure 3).

Body performance parameters of different chicken groups

The average weight gain (AWG) across the various treated groups was nearly similar before the challenge. Challenged NDV genotype VII significantly impacted AWG, as unvaccinated unchallenged G1 demonstrated significantly higher AWG at days 35 and 42 than the challenged unvaccinated G5. Different vaccination strategies also had a considerable influence on AWG, with vaccinated birds in groups G3, G4, and G2 showing higher values compared to the positive control G5 (p<0.05) at 35 days, respectively (Figure 4). Furthermore, vaccination led to a remarkable increase in average daily gain (ADG) (p=0.0005), with the ADGs for the unchallenged vaccinated groups G2, G3, and G4 achieving rates of 65.57, 63.44, and 66.39, respectively, compared to negative control G1 was 57.66.

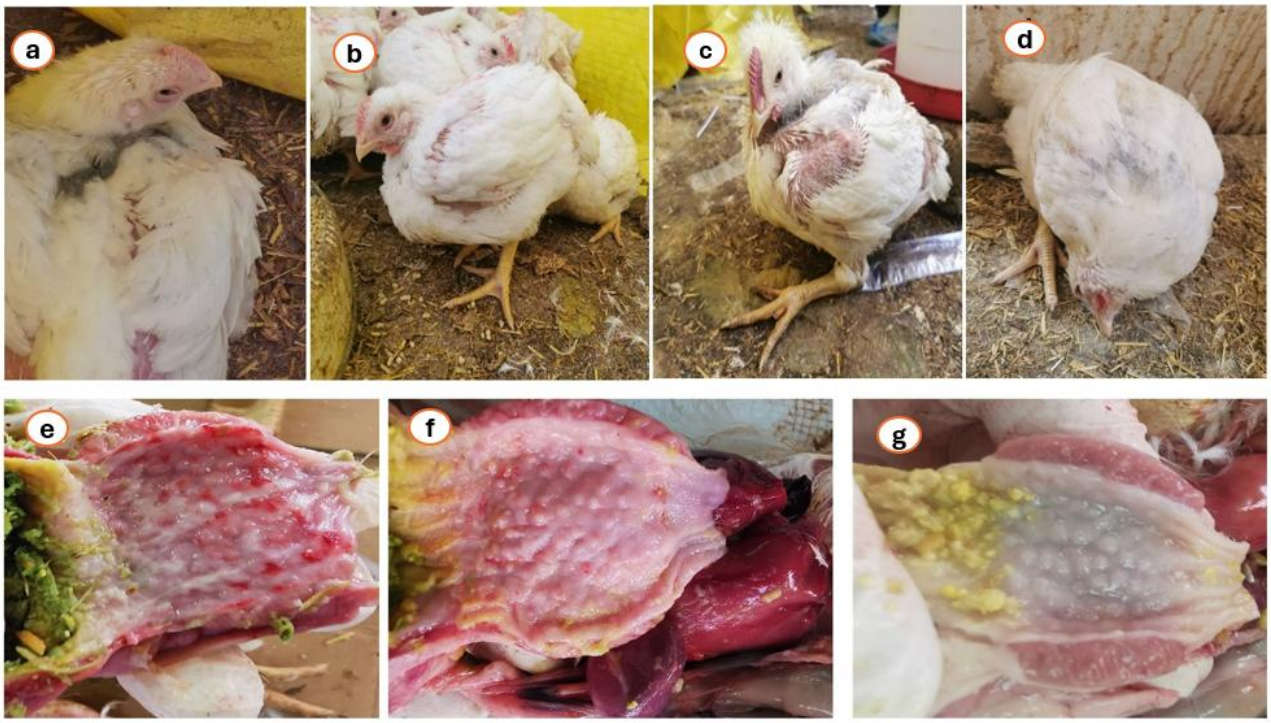

Figure 2: Clinical signs and lesions of commercial broiler chickens (Cobb) challenged with NDV. Broiler chicks at 5 days pch in unvaccinated challenged (G5) exhibited signs of conjunctivitis (a), greenish diarrhea (b), a recumbent posture (c), and neurological symptoms (d). The proventriculus gland at 6 days pch exhibited petechial hemorrhages in the non-vaccinated challenged group G5 (e). In contrast, it decreased in the vaccinated challenged group G3 (f) and was normal in the non-vaccinated non-challenged group G1 (g).

Table 1: Clinical manifestations were observed in various groups following the challenge with NDV genotype VII.

Groups	Depression	Conjunctivitis	Ocular discharge	Rales	Greenish diarrhea	Recumbent posture	Nervous signs
G1	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)
G2	5/15 (33.33%)	8/15 (53.33%)	4/15 (26.66%)	5/15 (33.33%)	6/15 (40%)	5/15 (33.33%)	1/15 (6.66%)
G3	10/14 (71.43%)	10/14 (71.42%)	4/14 (28.57%)	6/14 (42.85%)	9/14 (64.28%)	6/14 (42.85%)	2/14 (14.25%)
G4	5/15 (33.33%)	6/15 (40%)	3/15 (20%)	3/15 (20%)	5/15 (30%)	4/15 (26.66%)	1/15 (6.66%)
G5	20/25 (80%)	17/25 (68%)	5/15 (33.33%)	8/25 (32%)	20/25 (80%)	10/25 (40%)	5/25 (20%)

Chickens kept in G2, G3, and G4 were vaccinated subcutaneously with HVP360, vFP96, and HVP360+vFP96 at one day old, respectively. At 28 days of age, groups G2 to G5 were challenged intraocularly with 10^6 EID_{50} velogenic NDV genotype VII.

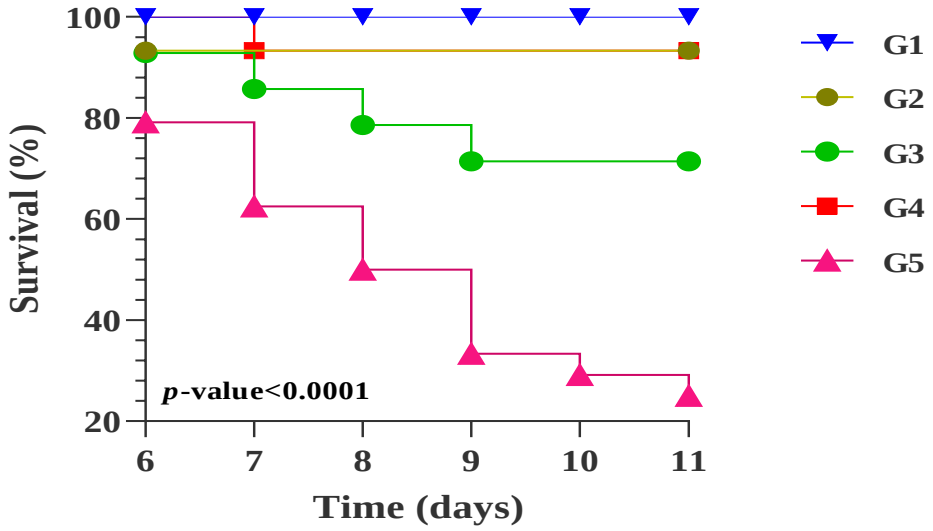

Figure 3: Survival rates of broiler chicks from different vaccinated groups following the NDV genotype VII challenge. Chickens kept in G2, G3, and G4 were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively. At 28 days of age, G2 to G5 were challenged intraocularly with 10^6 EID_{50} velogenic NDV genotype VII.

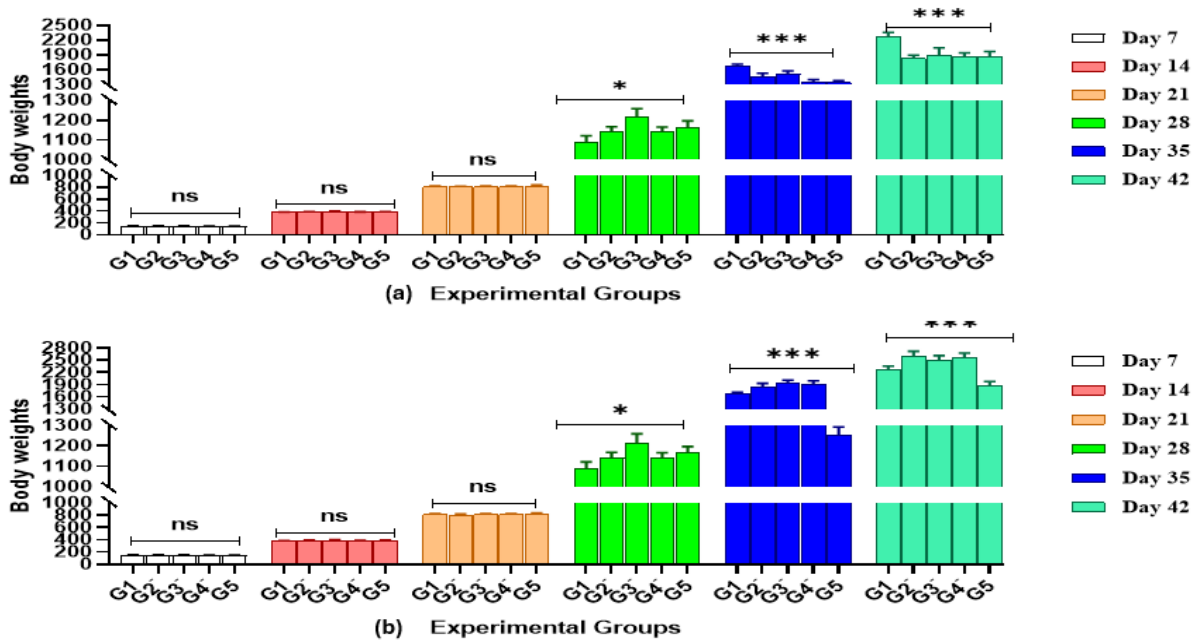

Figure 4: Average weight gain±standard deviation (SD). Chickens kept in G2, G3, and G4 were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively. G2, G3, and G4 were divided into G2, G3, and G4 (vaccinated-challenged) and G2-, G3-, and G4- (vaccinated-unchallenged). a) vaccinated-challenged groups compared to the negative control G1. b) vaccinated-unchallenged groups compared to the positive control G5. ns= not significant, *=p<0.05, ***=p<0.0001.

Serum antibody response following vaccination

Blood samples were collected weekly from all groups starting from day one of life to analyze the dynamics of the NDV serological response over 42 days, utilizing HI and ELISA tests. Significant differences were observed among the experimental challenge groups on days 14, 21, and 35, with G3 exhibiting the highest antibody titers in both HI and ELISA tests (Figure 5). In unchallenged groups, the antibody titers for the vaccinated groups G2, G3, and G4 showed a significant increase compared to the unvaccinated control G1, particularly on day 42 (Figure 6). Among the vaccinated groups, G4 achieved the highest antibody titers as measured by the ELISA assay, followed closely by G2.

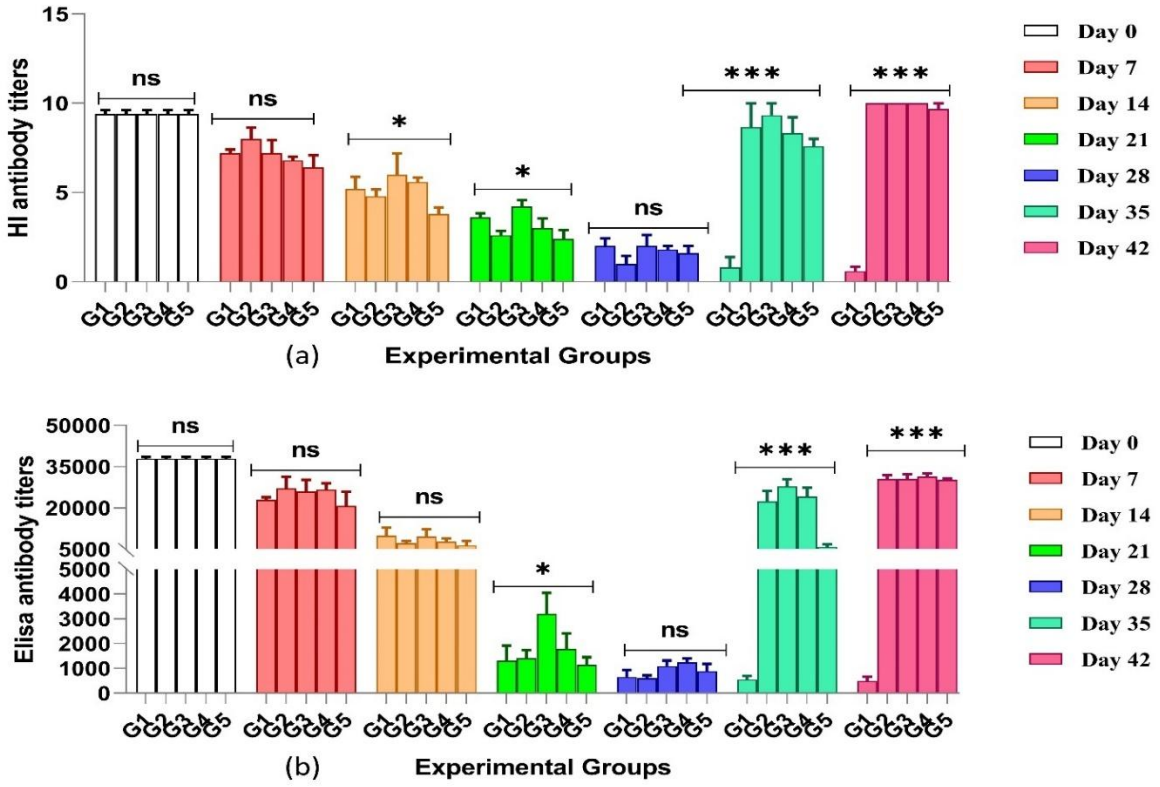

Figure 5: Serocnoversion in vaccinated-challenged groups compared with control. a) HI titers (means±SD using NDV genotype II antigen. b) ELISA antibody titers (means±SD) using F antigen. Chickens kept in G2, G3, and G4 were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively. G2 to G5 were challenged at 28-day-old intraocularly with 10^6 EID_{50} velogenic NDV genotype VII. ns= not significant, *=p<0.05, ***=p<0.0001.

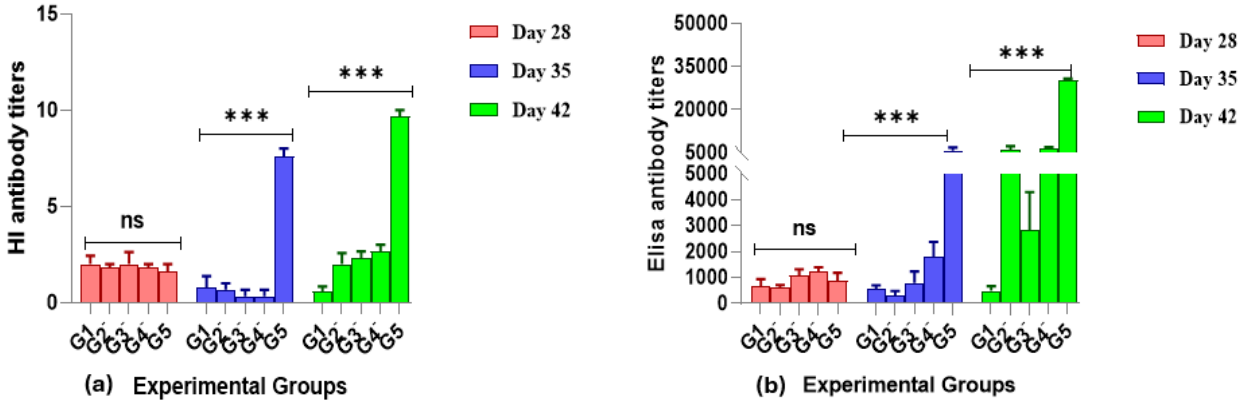

Figure 6: Seroconversion in vaccinated-unchallenged groups. a) Mean HI titers ± SD of different non-challenge groups compared with positive control G5 using NDV genotype II antigen. b) Mean ELISA antibody titers±SD of different non-challenge groups compared with positive control G5 using F antigen. Chickens kept in $G2^-$, $G3^-$, and $G4^-$ were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively.

Virus shedding following challenge

All experimental birds underwent examination for viral shedding through RT-PCR, utilizing tracheal and cloacal swabs collected on days 3, 5, 7, and 14 days pch. The negative control group, G1, exhibited no viral shedding throughout the trial. Notably, there were statistically significant increases (p<0.05) in virus counts from tracheal swabs when comparing the unvaccinated challenged G5 to all other challenged vaccinated groups on all days pch assessed, particularly on days 5 and 7. Meanwhile, vaccinated challenged G4 demonstrated the lowest counts among the groups (p>0.05) on days 5 and 7 pch, followed by G2 and G3, respectively. Viral shedding in cloacal swabs recorded the lowest rate on day 7 pch compared to other vaccinated groups (Figure 7a, b).

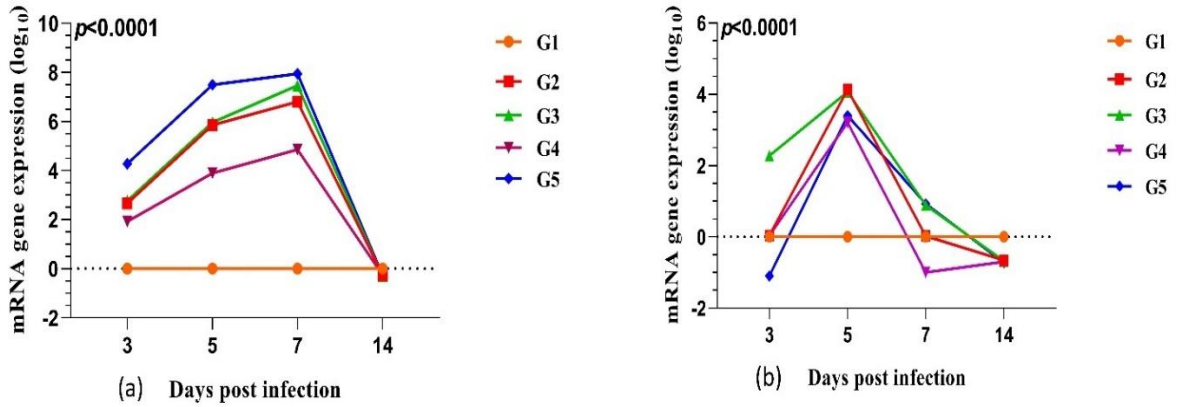

Figure 7: Virus shedding. a) Tracheal shedding titers of different vaccinated challenge groups compared with negative control G1. b) Cloacal shedding titers of different challenge groups compared with negative control G1. Chickens kept in G2, G3, and G4 were vaccinated subcutaneously at one day old with HVP360, vFP96, and HVP360+vFP96, respectively. At day 28 of age, G2 to G5 were challenged intraocularly with 10^6 EID_{50} velogenic NDV genotype VII.

Discussion

Newcastle disease poses a major risk to the poultry sector, leading to considerable economic and industry losses. Various factors have been associated with outbreaks of ND globally, such as insufficient biosecurity measures, poor vaccination, and immunization practices, antigenic variation, the interference of maternal antibodies with live vaccines, and immunosuppression (Chumbe et al., 2017; Dimitrov et al., 2017a).

Vaccination initiatives that rely on conventional vaccine programs don't adequately manage Newcastle disease infection in high endemic areas and may result in recurrent outbreaks of ND, leading to considerable viral shedding and significant economic consequences (Fawzy et al., 2020). Utilizing vector vaccines is an effective approach to address the limitations of conventional vaccination and achieve the following succeeding objectives: not interfering with maternal antibodies, inducing both humoral and cell-mediated immunity, protecting chickens from lethal NDV strains, and reducing virus shedding (Esaki et al., 2013). These vectors have demonstrated significant effectiveness in managing several critical diseases within the poultry sector, including Marek's disease and Gumboro disease (van Hulten et al., 2021). Recent studies have thoroughly examined its effectiveness in managing ND, especially following ND-VII outbreaks, despite rigorous vaccination efforts; however, all researchers have assessed its impact alongside live and/or inactivated ND vaccines (Ferreira et al., 2020; Ghanem et al., 2023), and its impact effect has not yet been investigated independently until now in Egypt. The objective of this study was to assess the protective effectiveness of various recombinant vaccines utilized in the poultry industry for ND, either HVT-based vector vaccines (HVP360) or fowlpox-based vaccines (vFP96.5), individually and in combination, without the inclusion of any supplementary live or inactivated ND vaccines.

Our clinical outcomes varied among the experimental groups in terms of their level of protection. The negative control G1 exhibited no clinical signs or lesions, ensuring that the experimental conditions were appropriate and that any extraneous variables that could influence the results were eliminated. In contrast, 80% of the birds in the positive control group G5 experienced green diarrhea and signs of depression, 68% developed conjunctivitis, approximately 33% showed rales and ocular discharge, and the survival rate was the lowest at 25%, which aligns with expectations for the unvaccinated challenged group (Ayoub et al., 2019; Moharam et al., 2019). In unvaccinated or vaccinated infected flocks that were not properly vaccinated, neurological symptoms observed following recovery from respiratory and gastrointestinal diseases have been consistently linked to velogenic NDV, as evidenced in the surviving birds in unvaccinated challenged-G5 and vaccinated vFP96.5-G3 (Sedeik et al., 2019; Nagy et al., 2020). The dual vaccinated challenged HVP360+vFP96.5-G4, followed by HVP360-G2, exhibited milder symptoms than

those in vFP96.5-G3.

Furthermore, they achieved the highest survival and protection rate at 93.33%. This finding aligns with the research conducted by van Hulten et al. (2021), indicating that the dual vaccination of HVP360 and vFP96.5-G4 and HVP360-G2 offered significant advantages and enhanced clinical protection that may be attributed to the possibility that HVT viral vector vaccine can bypass maternal antibody interference and establish a persistent infection that provides lifelong immunity (Calnek, 2001; Pan et al., 2022; Wang et al., 2024). In addition, the remarkable quick replication of the HN-NDV protein within the recombinant Fowl pox vaccine significantly enhanced the ELISA titers and facilitated an early immune response (Zhao et al., 2020). In contrast, vFP96.5-G3 alone offered a satisfactory protection level, reaching 71%. Zhao et al. (2020) reported a similar protection level of 66.7% against mortality and morbidity following NDV challenge when utilizing the rFPV-ND vaccine, which may be due to the interference of poxvirus with maternal immunity to some extent (Iritani et al., 1991; Wang et al., 2024).

Regarding gross lesions, the dead birds in the positive control G5 exhibited typical characteristic gross lesions associated with velogenic ND infection, including congested tracheitis, ulcers in the small intestine, hemorrhage at the tips of proventriculus glands and ileocecal tonsils. In contrast, less severe gross lesions were noted in all vaccinated groups G4, G2, and G3, respectively, confirming the effectiveness of the vaccination in protection against the challenge (Hines and Miller, 2012; Suarez et al., 2020). The decrease in the severity of symptoms and lesions observed with the use of recombinant vaccines across all vaccinated populations can be attributed to the vaccines' ability to avoid adverse side effects, their low risk of virulence return, and their commendable genetic stability (Esaki et al., 2013; Hein et al., 2021).

Newcastle disease infection is recognized for its impact on reducing bird weights due to diminished feed consumption (Alexander and Senne, 2008; Rehman et al., 2021). Consequently, the average weekly weight gain was a critical parameter in our evaluation. Our findings indicated a decline in average weight gain to 1229.65 grams by day 35, seven days pch, in the infected unvaccinated group G5, where the ADW was recorded at 46.82 grams. In contrast, the negative control G1 (unvaccinated and unchallenged) exhibited an average weight gain of 2282.95 grams on day 35 and an ADW of 57.66 grams. This aligns with the observations made by (Hines and Miller, 2012), which may be attributed to the fact that ND infection adversely affects both the exocrine and endocrine functions of the pancreas, which in turn hampers digestion and weight gain (Rehman et al., 2021). Vaccination showed a significant enhancement in AWG, with the values for challenged vaccinated chickens in groups G3, G4, and G2 respectively surpassing those of the positive control group G5 by day 35 ($P<0.05$) (Hines and Miller, 2012). The highest AWG was observed in the vaccinated vFP96.5-G3 group, which could be linked to the reduced number of chicks remaining after mortalities from the challenge, allowing the surviving chicks to consume more feed. Additionally, the unchallenged vaccinated groups G2, G3, and G4 demonstrated significantly higher ADG rates than negative control G1, with respective values of 65.57, 63.44, 66.39, and 57.66 gm, respectively. These results align with the findings of (Ellakany et al., 2018; Magdy et al., 2022).

Concerning the monitoring of humoral antibodies, the HI testing conducted on the unchallenged groups demonstrated a steady decline, ultimately resulting in adverse outcomes by 3 weeks of age. These findings align with those reported in earlier studies (Bertran et al., 2018). Conversely, the challenged group exhibited a gradual decline in MDA levels over time, reaching a peak one-week pch, where elevated titers correlated with immune responses to the challenge viruses. Our findings align with those of a previous report (Ghanem et al., 2023), which noted that birds vaccinated with the rHVT ND-IBD vaccine at five weeks of age had undetectable HI titers before the challenges. This is also consistent with the observations of another report (Iritani et al., 1991), which reported that antibody production against NDV was not detected in chickens vaccinated with the recombinant FPV-NDVHN. Measuring HI antibodies is widely recognized as a standard approach to assessing the protective efficacy of ND vaccines (Kapczynski and King, 2005). However, our findings do not align with this approach, and we suspect a special role of recombinant vaccines in eliciting different forms of immunity (Ingrao et al., 2017; Ingrao et al., 2018), which indicates a necessity

to incorporate alternative monitoring techniques, such as cellular immunity, particularly HVT vectors predominantly affect such aspects of an immune response. This view contradicts claims made by (Morgan et al., 1993; Ferreira et al., 2020), who argued that HVT is known to sustain antigen presentation, thereby maintaining or even enhancing the antibody response over time. The indirect ELISA-specific F protein test revealed a swift rise in antibody titers within one week following a challenge, particularly in the vaccinated groups G3, G4, and G2, which recorded significant titers of 27,867, 24,089, and 22,435, respectively.

In contrast, the challenged positive control group G5 had a titer of only 5,847. This outcome underscores the effectiveness of these vaccines in enhancing the immune response, aligning with the prime-boost strategy, which is in agreement with the research conducted by (Hossain et al., 2023), who compared groups that had been previously primed with those that had not. The unchallenged groups G4, G2, and G3, exhibited significant increases in antibody titers of 6,512, 5,978, and 2,835 respectively, at week 6 post-vaccination, which highlighted that G4 had a superior titer advantage within the vaccinated groups which that aligns with the results reported by (Ghanem et al., 2023), while G1 unvaccinated control displayed negative titers, corroborating the observations of (Ghanem et al., 2023) who noted improved titers obtained when various types of vaccines were administered.

Preventing the spread of the virus from infected birds is key to controlling ND infection (Kapczynski and King, 2005; Khan et al., 2010). Therefore, the amount of viruses vaccinated birds shed into the environment after exposure could be a valuable measure of vaccine efficacy. In this study, tracheal shedding assessed through qRT-PCR indicated that the positive control group G5 exhibited 100% virus shedding at 3, 5, and 7 days pch, confirming the virulent nature of the challenge virus (Sultan et al., 2021). Administration of the recombinant vaccine led to a significant reduction in viral shedding across all vaccination groups G2, G3, and G4 when compared to the positive control G5, which attributed to the recombinant vaccine possessing a low likelihood of horizontal transmission (Esaki et al., 2013; Hein et al., 2021), underscoring the effectiveness of the recombinant ND vaccine in minimizing viral shedding that consistent with findings from (Dimitrov et al., 2021). Birds in group G4, which received the HVP360+vFP96.5 vaccine, demonstrated a significant reduction in virus shedding, recorded as Log_{10} 1.93, 3.89, 4.85 in contrast to G2 (HVP360) with values of 2.66, 5.85, and 6.80, and G3 (vFP96.5) with 2.77, 5.96, and 7.47 at days 3, 5, and 7 pch, respectively. This enhanced reduction may be attributed to the combined vaccine approach, where the recombinant FPV improves immune protection and reduces viral shedding by expressing the F and HN proteins of the ND virus (Taylor et al., 1996). Additionally, the HVT360 vaccine effectively expresses the F protein of the ND virus across various organs due to the invasiveness of Marek's virus, contributing to long-lasting immunity (van Hulten et al., 2021), as well as the co-expression of cytokines and other immunomodulatory factors that promote cell-mediated immunity such as CD4 and CD8 alongside the humeral response has also been highlighted (Ingrao et al., 2017; Ingrao et al., 2018; Wang et al., 2024). Consequently, reductions in clinical signs, the decline in postmortem lesions, and damage of internal organs, besides the elimination of viral shedding, are key parameters utilized by researchers to evaluate the ND vaccination program, which has been proven to be significantly reduced in this study.

Conclusion

Newcastle disease virus genotype VII presents a significant challenge to the poultry sector, resulting in elevated morbidity, pronounced clinical symptoms, and mortality rates that can reach 75%. The use of recombinant ND vaccines has demonstrated improved clinical protection and a decrease in the severity of lesions, which has led to a notable reduction in NDV shedding among broiler chickens infected with virulent NDV VII. The strategic combination of the HVP360 strain and FP96.5 strain in combating the ND virus marks a pivotal advancement and can establish a foundational approach for vaccination programs targeting one-day-old chicks. Further investigation is needed to evaluate the cellular immune response to recombinant vaccines in conjunction with the antibody titers against the NDV to optimize vaccine efficacy.

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