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EFFECTIVENESS OF SINGLE AND DOUBLE RECOMBINANT HERPES VIRUS OF TURKEY VECTORED VACCINES BOOSTERED WITH LIVE ND VACCINE AGAINST THE HIGHLY PATHOGENIC NEWCASTLE DISEASE VIRUS GENOTYPE VII

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ABSTRACT

This study assesses the effectiveness of recombinant Newcastle disease vaccine formulations in one-day-old specific pathogen-free (SPF) chicks, with an emphasis on their performance against the current circulating NDV strain (Egypt-NDV-RLQP-2021) in Egypt. A total of 100 SPF chicks were divided into five groups; Group 1 received INNOVAX-ND-IBD®, Group 2 was given Vaxxitek ® HVT + IBD + ND, and Group 3 was vaccinated by Vectormune® ND, which utilized the herpesvirus of turkeys (HVT) as a vector by integrating the fusion (F) gene from Newcastle disease virus (NDV) into the HVT genome. Vaccinated groups received a single dose of live clone NDV genotype II vaccine at 15 days of age via intraocular instillation. Group 4 was kept as the positive control (no vaccination, challenged with NDV), and Group 5 was the negative control. Groups 1, 2, 3, and 4 were challenged 28 days later with virulent NDV genotype 7 with 0.2 mL 10^6 EID_{50} of a challenge virus intramuscularly. Chicks were monitored for clinical signs, mortality, and viral shedding using real-time PCR. Histopathological findings proved that all vaccinated groups showed good control in masking and preventing lesions compared to the positive control group 4. Serology examinations indicated a significant increase in antibody titer post-vaccination and challenge in all vaccinated groups, giving a protective immunity against NDV. For viral shedding, Group 2 showed no detectable virus at any time, which means superior control for viral shedding. In conclusion, our study highly recommends HVT-based vaccines together with NDV live vaccines, as they exhibited full clinical protection against NDV genotype VII.

Keywords: Newcastle, Vector vaccine, Genotype VII, SPF chicks.

INTRODUCTION

Newcastle Disease (ND), caused by the Newcastle Disease Virus (NDV), continues to be a major challenge for the global poultry industry (Alexander, 2000). This highly contagious viral disease affects various poultry species, including chickens and turkeys, and can lead to high mortality and economic losses, decreased egg production, and diminished growth performance in affected birds (Ahmed *et al.*, 2017). Therefore, the disease is notifiable (OIE, 2012).

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Newcastle disease virus (NDV), classified under the genus Avulavirus in the family Paramyxoviridae, is a negative-sense, single-stranded RNA virus characterized by a non-segmented genome that comprises six structural proteins: nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), and polymerase protein (L). It also produces two non-structural proteins, V and W (Mayo, 2002; Miller & Koch, 2013; Murulitharan *et al.,* 2013).

The clinical manifestations of ND ranged from mild respiratory symptoms to severe neurological signs and high mortality, which led to significant financial losses, especially in broilers. Also, the outbreaks affect both vaccinated and non-vaccinated chickens' houses (Amin *et al.,* 2022). Effective management of Newcastle Disease (ND) outbreaks relies heavily on vaccination, with various vaccine formulations available, including live attenuated and recombinant types (Aini, 1990; Alexander, 2000). Historically, live attenuated vaccines have been the cornerstone of ND control strategies. These vaccines, such as ND live colon, are favored for their ability to induce strong and long-lasting immunity (Moharam *et al.,* 2019).

HVT is a naturally apathogenic virus and is considered very safe for use in birds. It stimulates both humoral and cellular immunity, providing lifelong protection. Additionally, HVT can effectively overcome maternally derived antibodies and could accept foreign gene insertions at multiple sites (Gergen *et al.,* 2019; Naeem, 2023). Recombinant vaccines that express the hemagglutinin-neuraminidase (HN) or fusion protein (F) of NDV have been developed using various viral vectors. Several of these recombinant viral vaccines have received approval for use in poultry (Jia *et al.* 2022). Recombinant turkey herpesvirus (HVT) vector vaccines, especially those targeting Newcastle Disease Virus (NDV) or avian influenza strains, offer substantial benefits for poultry health. They are noted for inducing robust cellular immunity, allowing persistent immunity without adverse respiratory effects, and effectively reducing mortality and viral shedding. HVT-vectored vaccines are also compatible with day-old *in ovo* administration, simplifying early life immunization (Lee *et al.,* 2024).

Using a vector vaccine (expressing the F protein of genotype VII) with genotype II vaccines (live alone or live and killed) provides better protection based on clinical symptoms, mortality rate, severity of histopathology, and reduction in NDV shedding. (Sultan *et al.,* 2024).

This study aimed to evaluate and compare the efficacy of various (rHVT) NDV vaccines, which were boostered with a single dose of live attenuated NDV vaccine in one-day-old specific pathogen-free (SPF) chicks against the currently circulating strain of Newcastle Disease Virus in Egypt (Egypt-NDV-RLQP-2021).

MATERIALS AND METHODS

Ethics statement

The experiment was conducted in accordance with the recommendations of the animal welfare committee, following the approval of the protocols by the Research Ethics Board at the Faculty of Veterinary Medicine, Benha University, and in alignment with national guidelines. (No.: BUFVTM 06-04-23).

Viruses and vaccines:

The challenged Newcastle disease virus Genotype VII reference strain (Egypt-NDV-RLQP-2021) (MZ409479) was kindly provided by the Animal Health Research Institute, Dokki, and Giza. Birds were challenged with 0.2 mL of 10^6 EID_{50} of a challenge virus intramuscularly, according to the OIE, 2021.

The following commercially available recombinant ND vaccines were used in accordance with the manufacturers' guidelines:

- The recombinant HVT double vector vaccine, in market trade name Innovax®-ND-IBD (strain HVP360), encodes both the IBDV VP2 genes and NDV-F. S/C injection, one day old injection of 0.2 ml.
- The recombinant HVT in market trade name Vaxxitek HVT + ND + IBD (strain HVT310), encodes the F protein from genotype VII. S/C injection, one day old injection with a dose of 0.2 ml.
- The recombinant HVT vector vaccine (marketed as Vectormune® ND Ceva Santé Animale) is a recombinant HVT vector vaccine, which uses the herpes virus of turkeys (HVT) as the vector by inserting the fusion (F) gene from Newcastle disease virus (NDV) into the HVT genome. S/C injection, one day old injection with a dose of 0.2 ml.

-BIO-VAC CLONE – FATRO is a live vaccine against Newcastle Disease in chickens composed of: Live ND strain Clone 30: ≥ 6.0 log10 EID_{50}, used at 15 days of age, intraocular instillation.

Birds

Specific pathogen-free (SPF) chicks were obtained from the SPF egg project at Nile SPF Kom-Oshim, located in El-Fayoum Province, Egypt, under the Agricultural Research Center of the Ministry of Agriculture and Land Reclamation. All chicks were raised under controlled conditions in chicken isolators, within the BSL3 facility of the Experimental Animal Center at the Animal Health Research Institute in Dokki, Giza, of similar size, with identical light regimes and management practices, and were provided with water and feed ad libitum.

Experimental design.

In this experimental design, one-day-old chicks were divided into five groups, each consisting of 20 birds, to evaluate the efficacy of different vaccination regimens against the Newcastle Disease Virus (NDV). Group 1 was vaccinated with INNOVAX-ND-IBD. Group 2 received VAXXITEK® HVT+IBD+ND, and Group 3 was vaccinated with Vectormune® ND. Group 4 served as a positive control, receiving no vaccination, but challenged with NDV. Group 5 was the negative control, neither vaccinated nor challenged. Vaccination for the vaccinated groups (groups 1, 2, and 3) with the live clone vaccine against Newcastle Disease was administered at 15 days old (Table 1).

The first 4 groups were challenged at 28 days old with NDV, which was recently isolated from Egypt.

Chickens were observed daily for clinical signs of disease, including respiratory distress, diarrhea, depression, ruffled feathers, edema, tremors, torticollis, and paralysis of wings and legs. Mortality rates were recorded throughout the study.

Table 1: Experimental design

Groups	Vaccination (on zero day)	Colone on 15 Day Old	Challenge (on 28th day old)
1	Innovax®- ND-IBD	√	√
2	VAXXITEC IBD-ND®	√	√
3	Vectormune ND®	√	√
4	N\A	N\A	√
5	N\A	N\A	N\A

N\A: Not Applicable

Sample collection

- Cloacal swabs were collected from each group at 3-, 5-, and 7-days post-challenge for quantification of NDV using real-time PCR.
- Serum samples were collected at 7, 14, 21, 28, 31, and 35 days of age for serological analysis and indirect ELISA, utilizing the F antigen to detect anti-NDV antibodies in the chicken sera.

Histopathology

On the 3rd, 5th, 7th, and 9th days post-challenge (DPC), three chicks from each group were humanely euthanized, and the trachea, lung, brain, cecal tonsils, proventriculus, kidney, and the bursa of Fabricius were collected. Tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E) for examination under light microscopy, as described by Bancroft and Gamble (2008).

Microscopic lesion scores for various organs were assessed using established criteria. For the proventriculus, the scoring system was as follows: 0= normal; 1= mild epithelial cell degeneration and necrosis with heterophils; 2= extensive epithelial cell degeneration and necrosis with mononuclear cell infiltration; 3= destruction of lymphoid areas, often accompanied by fibrin; 4= complete destruction of lymphoid areas with some hemorrhage (Mousa *et al.,* 2021).

Kidneys were scored as 0 = no changes; 1= few inflammatory cells infiltration in interstitial tissue; 2= inflammatory cells infiltration and focal degeneration in renal tubules; 3= inflammatory cells with foci of necrobiotic changes in renal tubular epithelium and/or glomerulopathy; 4= inflammatory cells, necrobiotic changes in medullary epithelium, and renal glomerulopathy (Mousa *et al.*, 2020).

Lung lesions were categorized as 0 = normal; 1= inflammatory cell infiltration in the air capillaries; 2 = inflammatory cell infiltration, hemorrhage, and exudate into the secondary bronchi; 3= hypertrophy of the tertiary bronchial epithelium and interstitial oedema (Mousa *et al.*, 2021).

Trachea lesions were assessed as 0 = normal; 1= hyperemia and inflammatory cells infiltration; 2= hyperemia, inflammatory cells infiltration, and oedema; 3 = hyperemia, inflammatory cells infiltration, oedema, and deciliation; 4= slight hyperplasia and deciliation; 5= hemorrhagic patches, desqua-mation, and hyperplasia (Mousa *et al.*, 2021).

Bursa of Fabricius lesions were scored as 0= normal; 1= mild (scattered follicles with mild necrosis); 2= mild to moderate (follicles with moderate to severe lymphoid depletion); 3= moderate (over 50% of follicles with severe lymphoid depletion); 4= moderate to severe (cysts in follicles, increased connective tissue, thickening, and folded epithelium); 5= severe (complete disruption of follicular structure and increased fibroplasia) (Mousa *et al.,* 2019).

Cecal tonsils lesions were categorized as: 0 = normal; 1= mild (very few proliferative lymphoid follicles); 2= moderate (many active lymphoid follicles); 3= severe (considerable dissemination of active lymphoid follicles or focal necrosis) (Mousa *et al.,* 2019). Brain lesions were assessed with scores: 0= normal; 1= perivascular infiltration of mononuclear cells; 2= endothelial hypertrophy and neuronal necrosis; 3= spongy change, gliosis, and hemorrhage (Hussein *et al.,* 2018).

Quantification of ND Challenged virus by real-time polymerase chain reaction

Total viral RNA was extracted from cloacal swabs using the QIAamp Viral RNA Mini Kit (QIAGEN) catalogue No. 52904 according to the manufacturer's instructions. QuantiTect probe RT-PCR (catalogue No. 204443) kits for quantitative NDV genome identification and quantification by RT-PCR. The primers and probes used in the reaction were provided by Metabion (Germany) **(Table 2).** Cycling conditions of the used primers and probe were as follows: primary denaturation at 95°C for 15 min, and then the secondary denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec, and final extension at 72°C for 10 sec were repeated for 40 cycles (Wise *et al.,* 2004).

Statistical analysis

Data was collected in a spreadsheet by using SPSS version 22. Each element and its contents, calculated by mean and standard deviation ($\bar{x}$±SD), were applied to the ANOVA test to display a one-way analysis-of-variance table within each group. Then followed by the Tukey HSD test, and at a level of 95% was considered statistically

significant, followed by the Monte Carlo test to predict the association between the independent variables and the dependent variable. Finally, to predict the effect of time during the study, the Kaplan-Meier curve was done at a level of 95%.

Table 2: Oligonucleotide primers and probes

Virus	Gene	Primer/ probe sequence 5'-3'	
ND	Matrix	**M+4100**	5'-AGTGATGTGCTCGGACCTTC-3'
		M-4220	5'- CCTGAGGAGAGGCATTTGCTA-3'
		M+4169	[FAM]TTCTCTAGCAGTGGGACAGCCTGC[TAMRA]-3'

RESULTS

Clinical signs and mortalities

All groups except the positive control showed no mortality. However, chickens of groups 1 and 2 exhibited notable clinical signs of neurological distress, including anorexia and ruffled feathers. These signs were observed consistently across all time points (3-, 5-, and 7-day post-challenge). All birds from the control challenged group showed mortality by 4-5 days post-challenge.

Histopathological lesions

Pathological findings of the Trachea:

Examination of the control revealed the normal histological structure of the tracheal mucous membrane (Fig. 1A). The mucosa was lined by pseudostratified columnar ciliated epithelium with several interepithelial mucous glands. Meanwhile, the control +ve group showed marked histopathological alterations, which were characterized by marked thickening of the tracheal mucosa with an increased number of mononuclear inflammatory cell infiltrations with congested blood vessels at 48 hours post-infection (PI). Some examined sections showed focal squamous metaplasia of the severely affected mucosal surface. At 5 DPI, diffuse ulceration and destruction of the tracheal mucosa with accumulation of necrotic tissue debris and severe deciliation (Fig. 1B). Group 1: The tracheal mucosa showed 5 DPI normal histological structures, except for some sections that revealed mild mucous exudates in the tracheal lumen mixed with desquamated epithelial cells. Some examined sections showed variable submucosal edema with fewer erythrocyte exudates. (Fig. 1C). Group 2: The tracheal mucosa appeared 5 DPI, mostly normal in most sections. Mild submucosal edema was observed in some birds, along with moderate tracheitis in a few individuals, characterized by mononuclear inflammatory cell aggregations, submucosal edema, and focal hemorrhagic areas. (Fig. 1D). Group 3: Moderate tracheitis was noticed in some affected individuals, characterized by a variable number of mononuclear infla-mmatory cell aggregations associated with submucosal edema and focal hemorrhagic areas (Fig. 1E). Tracheal histopathologic lesions score of different groups at 5 DPI. Values are reported as mean±SE. A significant difference was considered at $P<0.05$ (Fig. 1F).

Pathological findings of the Lung.

Microscopic examination of the control group revealed normal respiratory lobules, which consisted of air and blood capillaries that surround parabronchi (Fig. 2A). In the control positive group, marked congestion of blood capillaries and interstitial blood vessels was observed at 48 hours PI. By 5 DPI, the lungs displayed severe bronchitis, particularly the primary and secondary bronchi. The bronchial lumen contained mucous exudates, desquamated epithelial cells, and inflammatory cell infiltration. Interstitial tissue exhibited edema and hemorrhages. Proliferative responses included obliteration of atrial and infundibular spaces of the parabronchus by proliferating the lining epithelium, extending into the adjacent capillary bed, with

hypertrophied smooth muscles in the lining of severely affected parabronchi (Fig. 2B). Group 1: The pulmonary tissue appeared 5 DPI, mostly normal in several examined birds, with only sporadic cases showing congestion of interstitial blood vessels. (Fig. 2C). Group 2: The pulmonary tissue at 5 DPI was intact, with no significant histopathological alterations observed. The bronchi and air capillaries remained intact, and mild histopathological alterations were detected in some sections (Fig. 2D). Group 3: the pulmonary tissue at 5 DPI with intact bronchi and air capillaries, and the secondary bronchi, mild smooth muscle hypertrophy, and limited hyperplastic epithelial cells (Fig. 2E). Lung histopathologic lesions score of different groups at 5 DPI. Values are reported as mean±SE. A significant difference was considered at $P<0.05$. (Fig. 2F).

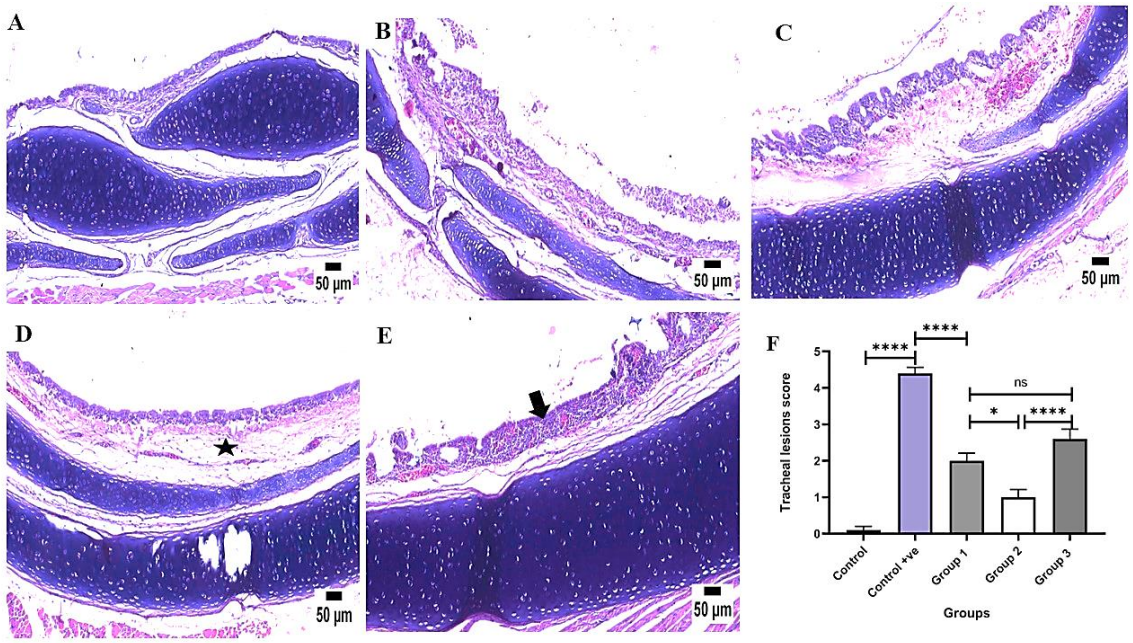

Fig.1: Histopathological changes in the trachea were observed with (H&E) stain at 5 DPI.
A-Photomicrograph of the trachea from the control group, showing normal histological structure B- The control positive group, displaying necrosis of the tracheal mucosa with an accumulation of necrotic debris in the tracheal lumen. C- Group 1, revealing submucosal edema with minimal erythrocyte infiltration. D- Group 2, exhibiting an apparently normal mucosal epithelium with mild submucosal edema. E- Group 3, indicating moderate tracheitis.

Pathological findings of Cecal tonsils:
Control Group: the cecal tonsils exhibited a normal histological structure, characterized by diffuse and nodular aggregations of lymphocytes (Fig. 3A). Control Positive Group: 48 hours PI: submucosal hemorrhages were frequently observed, accompanied by mild lymphoid depletion and lymphocytolysis in the lymphoid follicles. (Fig. 3B). All groups 1, 2, and 3 showed marked protection characterized by normal lymphocytes in the lymphoid follicles and diffused aggregations in the submucosal layer at 5 DPI (Fig. 3C, D, E). Cecal tonsils' histopathologic lesions score of different groups at 5 DPI. Values are reported as mean±SE. A significant difference was considered at $P<0.05$. (Fig. 3F)

Pathological Findings of the Brain
Control Group: the brain-stained sections showed a normal histological structure without any detectable alterations in the cerebral cortex (Fig. 4A). Control Positive Group: 48 Hours PI and 5 Days Post-Infection (DPI): Severe thickening of blood vessel walls, marked vasculitis, perivascular edema, and infiltration of inflammatory cells in the cerebral cortex were observed. Additionally, diffuse gliosis and neuronal

degeneration were detected (Fig. 4B). Regarding all vaccinated groups 1, 2, and 3 (Fig. 4C, D, E), marked protection was detected with no abnormalities observed and numerous scattered neurons in different brain regions in the cerebral cortex. Brain lesion score of different groups at 5 DPI. Values are reported as mean±SE. A significant difference was considered at P<0.05 (Fig. 4F).

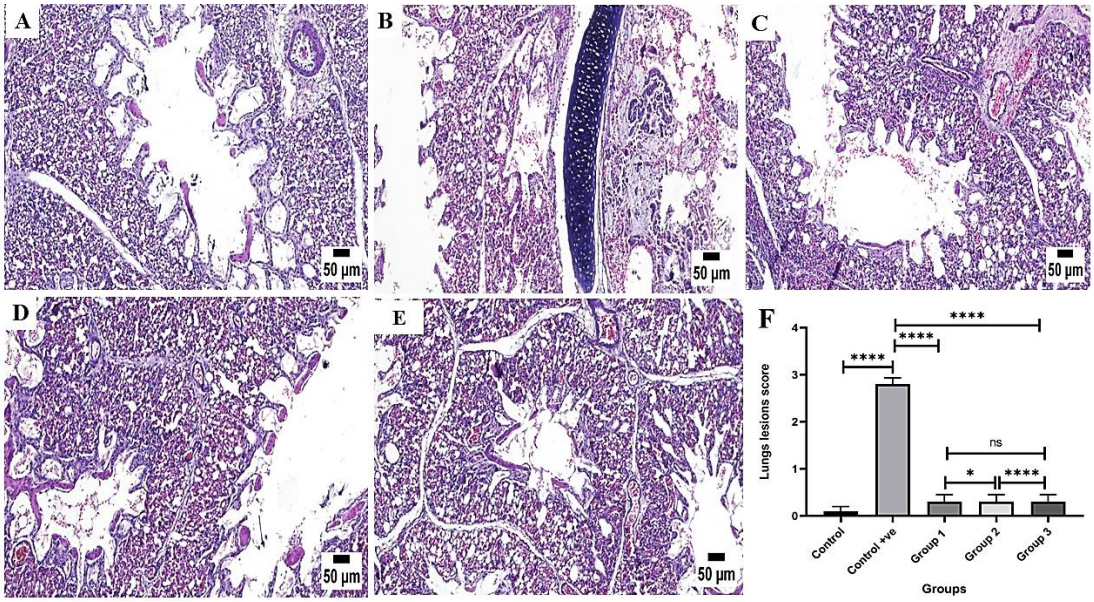

Fig.2: Histopathological changes in the lung with (H&E) stain at 5 DPI.

A- Photomicrograph of lungs, control group showing the normal histological structure of parabronchus, atrium, and infundibulum. B- control +ve group showing accumulation of mucous exudates, desquamated epithelial cells and hemorrhages in the lumen of primary bronchi (star). C- Group 1 showing normal parabronchi and healthy air capillaries. D- Group 2 shows normal respiratory lobules with intact air and blood capillaries that surround tertiary bronchi. E- Group 3 showing normal respiratory lobules.

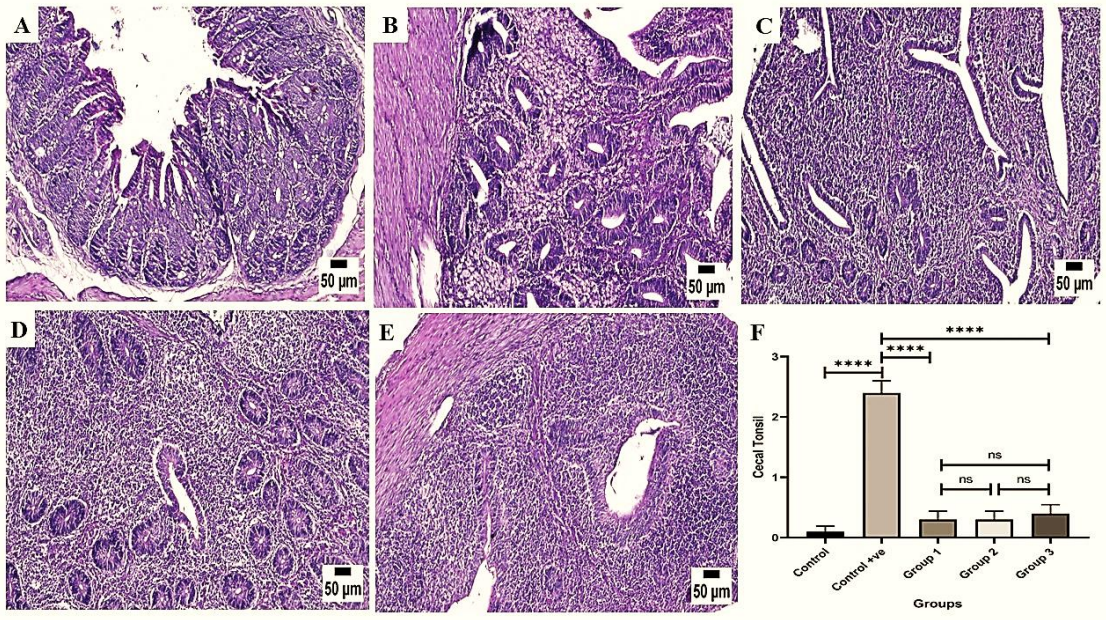

Fig. 3: Histopathological changes of cecal tonsils with (H&E) stain at 5 DPI.

A- control showing normal cecal tonsils. B- control +ve group showing many macrophages with cytoplasm distended with phagocytized debris. These macrophages give the cecal tonsils a vacuolated appearance (star). C- Group 1 showing normal cecal tonsils. D- Group 2 showing normal cecal tonsils. E- Group 3 showing normal cecal tonsils**.**

Pathological findings of the Bursa

Control Group: The bursa of Fabricius displayed a normal histological structure, featuring typical folds (plica) and numerous lymphoid follicles separated by thin bands of fibrous connective tissue (Fig. 5A).

Control Positive Group: 48 Hours PI: There was an expansion of the interfollicular connective tissue accompanied by edema, congested blood vessels, and minimal inflammatory cell infiltration.

At 5 DPI, severe alterations were observed, including increased folding of the plica, marked atrophy of the lymphoid follicles, lymphocytolysis, and necrosis. The bursal lymphoid follicles exhibited numerous vacuolated macrophages containing phagocytic debris. The interfollicular connective tissue was extensively expanded, with edema, hemorrhages, and a significant presence of inflammatory cells (Fig. 5B).

In the vaccinated groups (1, 2, and 3), all examined birds demonstrated a normal histological structure of the bursa of Fabricius, revealed normal plica covering intact lymphoid follicles (Fig. 5C, D, E). Bursa of Fabricius histopathologic lesions score of different groups at 5 DPI. Values are reported as mean ±SE. A significant diff-erence was considered at $P<0.05$ (Fig. 5F).

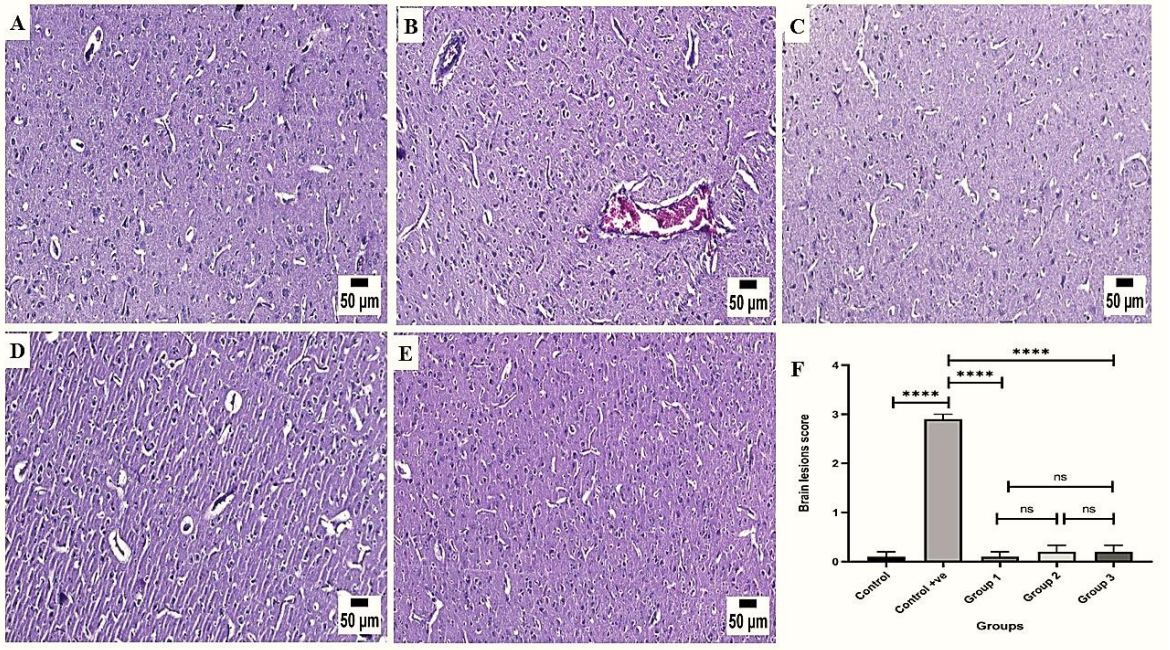

Fig. 4: Histopathological changes in the brain with (H&E) stain at 5 DPI.

A- Photomicrograph of the brain, control group showing the normal histological structure of neurons in the cerebral cortex. B- control +ve group at 5 DPI showing severely congested blood vessels in the cerebral cortex. C- group 1 shows the normal histological structure of neurons in the cerebral cortex. D- group 2 shows the normal histological structure of neurons in the cerebral cortex. E- group 3 shows a normal histological structure of neurons in the cerebral cortex.

Pathological findings of the Proventriculus:

Control Group: The proventriculus exhibited a normal histological structure of both the mucosa and glandular acini in all examined birds (Fig. 6A). 48 Hours PI: Multifocal infiltration of inflammatory cells was observed in the glandular acini and submucosal layer, accompanied by dilated blood vessels. At 5 DPI: Severe necrosis of the epithelial lining of the glandular acini, sloughing of the epithelial layer, congested blood vessels, and multifocal hemorrhagic areas were detected (Fig. 6B). Group 1, 2, and 3: Mild inflammatory foci and mild congestion of blood vessels were observed in the glandular acini. Several examined birds had apparently normal glands (Fig. 6C, D, E).

Proventriculus histopathologic lesions score of different groups at 5 DPI. Values are reported as mean±SE. A significant difference was considered at P<0.05 (Fig. 6F).

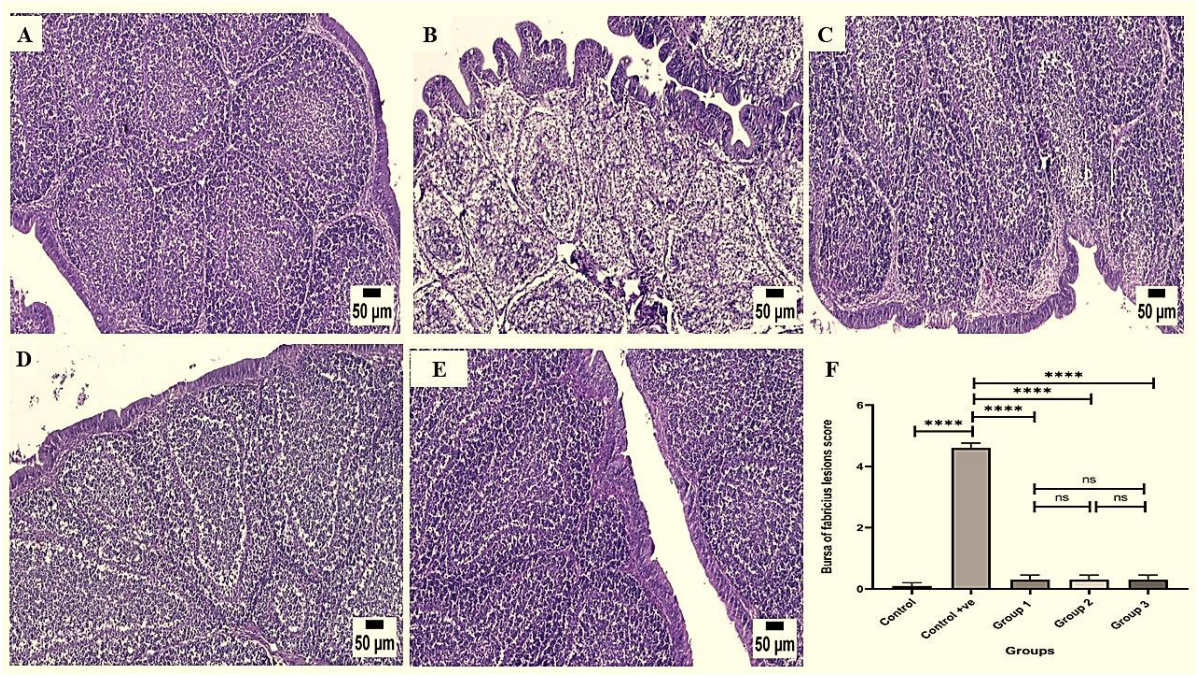

Fig. 5: Histopathological changes of Bursa with (H&E) stain at 5 DPI.
A- control showing normal bursa structure. B- control +ve group showing marked folding of the plica (arrow) with extensive damaged lymphoid follicles that contain numerous vacuolated macrophages. C- group 1 showing normal bursa structure. D- group 2 showing normal bursa structure. E- group 3 showing normal lymphoid follicles and plica epithelial cells.

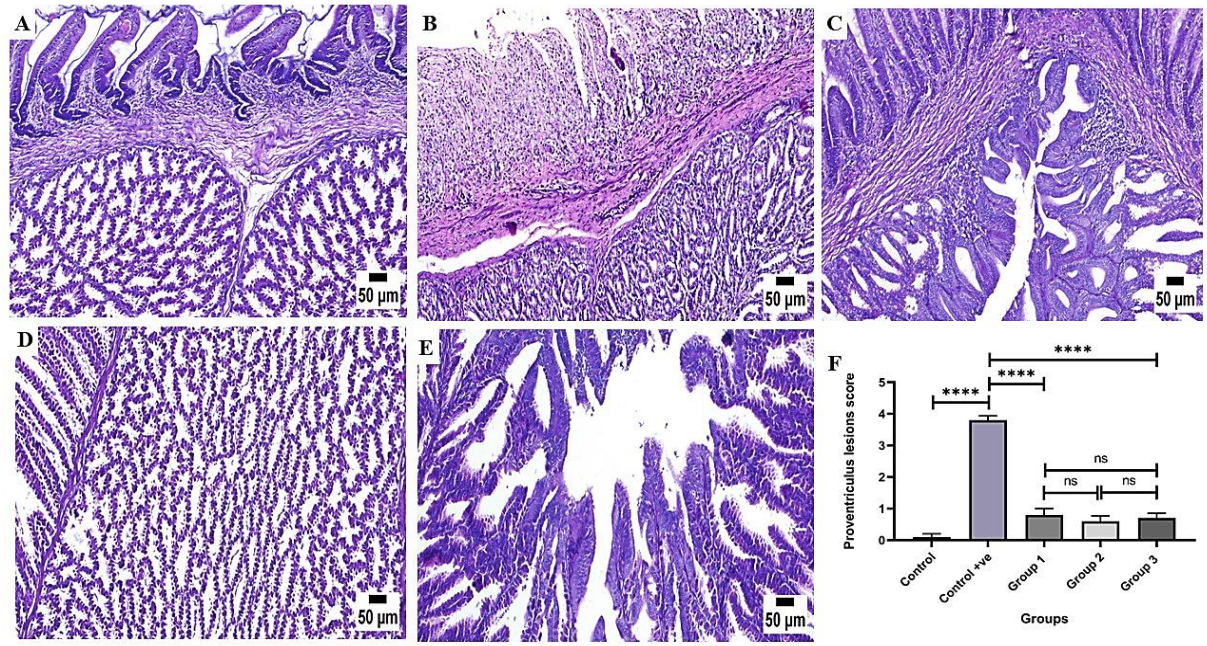

Fig. 6: Histopathological changes of the Proventriculus with (H&E) stain at 5 DPI.
A- Photomicrograph of proventriculus gland, control group showing the normal histological structure of mucosa and glandular acini. B- control +ve group showing severe necrosis of the mucosal covering (arrow), and necrosis of the glandular epithelium with numerous congested blood vessels (arrow), C- group 1 showing mild inflammation of the glandular acini (arrow). D- group 2 showing normal glandular acini. E- group 3 showing normal glandular acini.

Viral shedding

In this study, viral shedding was assessed through the quantification of the challenge virus in cloacal swab samples collected at various time points post-challenge.

- Group 1: the presence of virus shedding on the 5th day post-challenge (DPC) with moderate CT values and viral concentrations indicates partial protection, as the virus was still detectable. However, the absence of virus detection by the 3rd and 7th DPC suggests effective initial control and eventual clearance of the virus.

Group 2: The virus was once detected at tested DPCs, indicating strong protection against NDV. Group 3: Viral shedding was detected on the 5th and 7th day post-challenge (DPC) with moderate CT values and viral concentrations on both days, which means moderate protection. Group 3: Shedding was detected on both the 5th and 7th DPC. CT values ranged from 26.59 to 28.04 on the 5th DPC, with viral concentrations reaching up to 1.409 x 10^5 EID_{50}/ml. On the 7th DPC, CT values remained in a similar range, indicating sustained virus shedding. The positive control group consistently showed virus shedding across all days, with low CT values and very high viral concentrations, peaking at 9.435 x 10^7 EID_{50}/ml, confirming effective challenge exposure. Negative Control: No virus shedding was observed at any point. A one-way ANOVA was conducted to compare viral concentrations (EID50/ml) among group 1, group 3, and the positive control on the 5th DPC. The analysis yielded an F-value of 4.40 and a P-value of 0.067. Although the P-value is slightly above the 0.05 significance threshold, it suggests possible differences in virus shedding between the groups Fig (7).

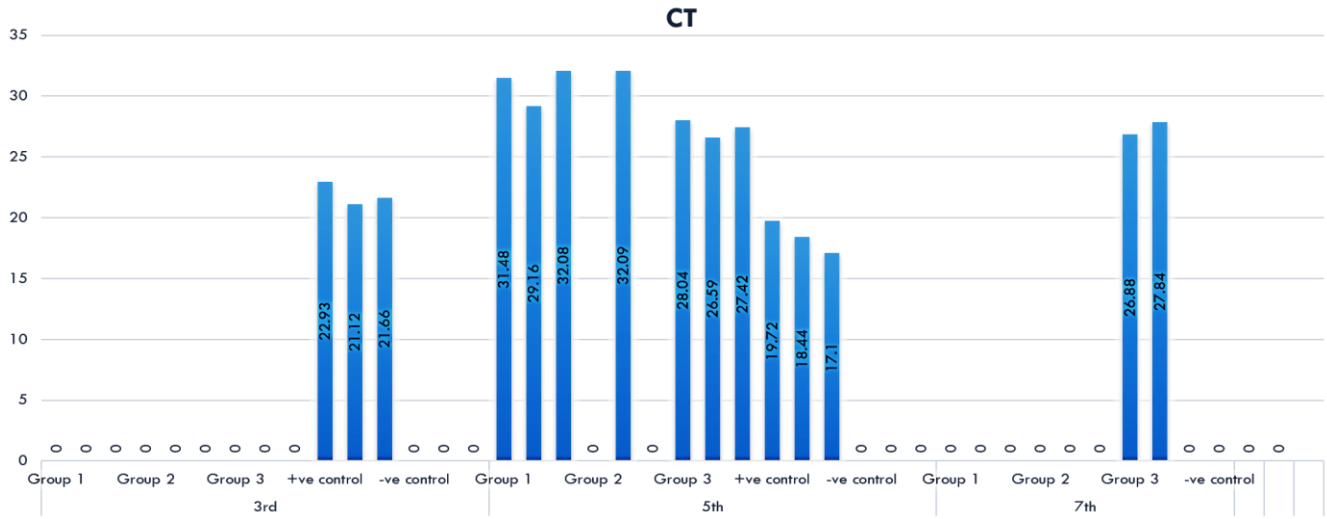

Fig. (7) the chart illustrating virus shedding post-vaccination showing the CT values

Serology:

Figure (8) presents the mean titers and standard deviations for different study groups measured on days 7, 14, 21, 28, 31, and 35 days of age.

-Day 7: Group 1 had a mean titer of 353.20 with a standard deviation of 198.601. The highest mean titer was observed in the Control +ve group (456.00), while the lowest was in Group 2 (236.8).

-Day 14: Group 1 showed a significant increase in mean titer to 1342.60, with a high standard deviation of 1292.214, indicating variability among subjects. Group 3 had the highest mean titer (1742.40) on this day.

-Day 21: Group 1 continued to increase in mean titer, reaching 3697.80, with the highest mean titer observed in Group 3 (4205.40).

-Day 28: Group 1 demonstrated a substantial rise in mean titer to 12443.40, though Group 3 had the highest mean titer (14613.00) with a standard deviation of 2848.150, indicating some variability.

-Day 31: The mean titer for Group 1 was 12972.40. The highest mean titer was observed in Group 3 (13009.60), closely followed by Group 1.

-Day 35: Group 1 showed a peak mean titer of 20097.00, with a standard deviation of 1139.264, indicating consistently high titers across subjects. However, Group 2 had the highest mean titer (20963.80) on this day.

The data shows an increasing trend in titers over days across all treatment groups. Group 1 demonstrates a gradual but substantial increase in titers, particularly after Day 14. By Day 35, it achieves one of the highest mean titers, indicating that it is highly effective in inducing a strong immune response over time.

The standard deviations, particularly in the earlier days, suggest some variability in response among subjects, which became more consistent by Day 35 (Table 3).

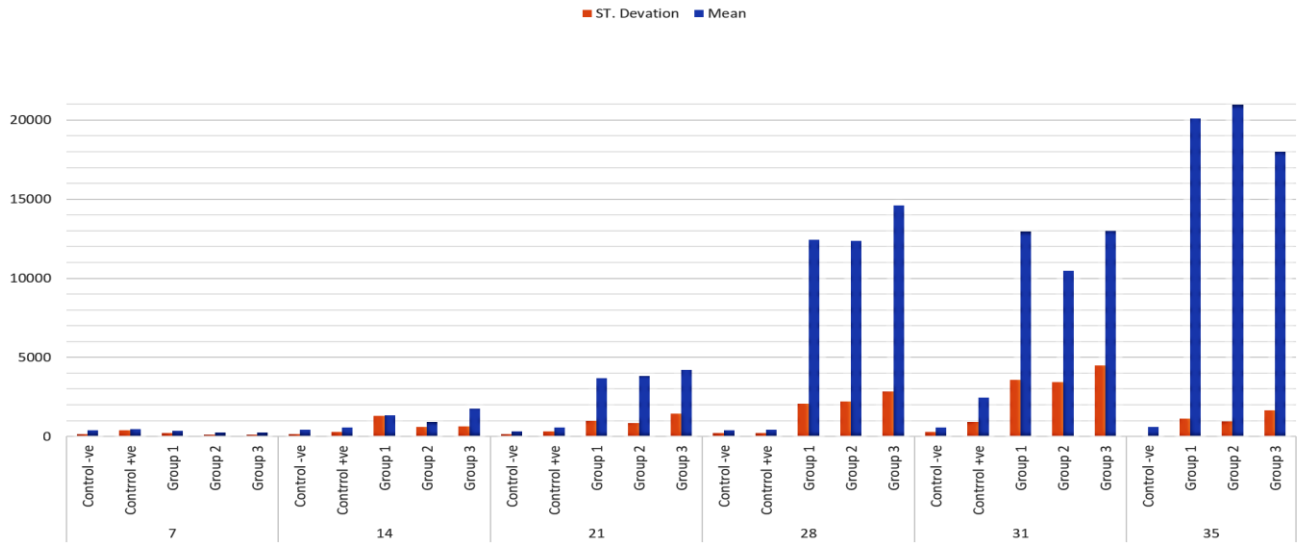

Fig. (8): the estimated marginal means of titers over time for different study groups

Table 3: pairwise comparisons of different groups

Measure time:						
(I) study group	(J) study group	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[s]	
					Lower Bound	Upper Bound
Control -ve	Control +ve	-797.100-	354.580	.360	-1915.232-	321.032
	Group 1	-8035.067	354.580	.000	-9153.199-	-6916.935-
	Group 2	-7681.633	354.580	.000	-8799.765-	-6563.501-
	Group 3	-8189.500	354.580	.000	-9307.632-	-7071.368-

DISCUSSION

Outbreaks of Newcastle disease (ND) have been linked to several factors, such as poor biosecurity programs, low-quality vaccines and vaccination regimes, antigenic variation, MDA interference with live vaccines, and immunosuppression problems (Chumbe *et al.*, 2017). Recent outbreaks in Egypt have been associated with Class II Genotype VII strains of NDV circulating in poultry farms across various governorates (Sultan *et al.*,

2024). The primary objectives of vaccination are to eliminate clinical disease, reduce the shedding of virulent viruses, and lower the infectious dose of challenge viruses (Kapczynski *et al.,* 2013).

Despite the implementation of numerous vaccination strategies using conventional vaccines, many ND outbreaks continue to occur, leading to significant viral shedding and economic losses due to mortality and decreased egg production (Fawzy *et al.,* 2020). This study aimed to assess the efficacy of various Newcastle Disease vaccines, particularly recombinant formulations, in one-day-old specific pathogen-free (SPF) chicks against NDV strains circulating in Egypt.

The objective was to evaluate whether an advanced vaccination approach, utilizing the herpes virus of turkey (HVT) as a backbone with multiple inserts such as the F gene of NDV and VP2 of IBDV, either alone or in combination with live vaccines, could enhance protection, decrease viral shedding, and potentially reduce the spread and transmission of the virus. The results indicated that the recombinant vaccines in Groups 1, 2, and 3 provided significant protection against NDV challenges, as evidenced by the absence of mortality in these groups. In contrast, the positive control group (Group 4) experienced high mortality within 4-5 days post-challenge, underscoring the virulence of the NDV strain employed in the study.

The findings of this study underscore the essential role of recombinant vaccines, particularly those utilizing the herpes virus of turkey (HVT), in controlling Newcastle Disease Virus (NDV) outbreaks in Egypt and similar areas. The demonstrated effectiveness of combining recombinant vaccines with live vaccines suggests a promising strategy for enhancing herd immunity and minimizing viral shedding in poultry populations (Fadly & Hatta, 2019; Abd El-Hamid & Soliman, 2020; El-Sayed & Eldeeb, 2021). This is especially crucial in regions where NDV genotype VII strains are widespread, as these strains are linked to increased virulence and substantial economic losses in the poultry industry (Sultan *et al.,* 2024).

Histopathological examinations were conducted on respiratory organs, cecal tonsils, the brain, bursa of Fabricius and proventriculus to assess the impact of the vaccines on different tissues. The evaluation of microscopic lesion scores revealed the potential of the vaccines to mitigate pathological changes associated with Newcastle disease. The histopathological examination of the trachea provides valuable insights into the impact of different Newcastle disease vaccines on the respiratory system. The control positive group experienced severe pathological changes, including ulceration, destruction of the mucosa, and necrotic debris accumulation, which agreed with Mousa *et al*. (2019), who found tracheal congestion, catarrhal tracheitis, and mucous exudation in the tracheal lumen in the non-vaccinated birds as well. The experimental groups, particularly group 1 and group 3, demonstrated marked protection with minimal histopathological alterations. The observed differences in lesion scores suggest that these vaccine group 2 contributed to varying levels of protection against Newcastle disease, while showing particularly promising results in maintaining the normal histological structure of the trachea even under viral challenge, which disagreed with the results of Sultan *et al.* (2024), who found the trachea displayed mucosal thickening, with lining epithelium hyperplasia and mucous glands activation, congested blood vessels and edema in mucosa and submucosa in the vaccinated group with recombinant ND vaccine. The control positive group exhibited severe lung, cecal tonsil, brain and proventriculus lesions, emphasizing the damaging effects of Newcastle disease. In our investigation, vaccinated groups provided high lung, cecal tonsil, brain, and proventriculus protection, maintaining normal histological structure, which indicates the excellent results of all

used vaccines to protect the mentioned organs from the Newcastle infection.

By implementing advanced vaccination strategies that integrate HVT-based vaccines with traditional live vaccines, poultry producers can mitigate the impact of NDV, promote better animal health, enhance productivity, and contribute to food security in regions that rely heavily on poultry farming (Kapczynski *et al.,* 2013; Ghanem *et al.,* 2023).

While the vaccines demonstrated effectiveness in preventing mortality, signs of neurological distress, anorexia, and ruffled feathers were noted in the vaccinated groups (Groups 1, 2, and 3) following the challenge. Although these symptoms were less severe than those observed in the unvaccinated and challenged group, researchers suggest the vaccines may not completely eliminate the possibility of subclinical infection or an immune response to the challenge virus. This observation is consistent with findings from Mousa *et al.* (2021), which reported significant clinical symptoms and mortality in broiler chickens infected with a virulent NDV genotype VII. Their study noted severe depression, greenish diarrhea, and pronounced respiratory sounds, leading to mortality rates as high as 24% in the infected groups (Mousa *et al.,* 2021).

Viral shedding was assessed through the quantification of the challenge virus in cloacal swab samples collected at various time points post-challenge.

The use of antigen-matched vaccines is effective for both inactivated and live vaccines developed from homologous challenge virus genotypes to increase efficacy against virulent challenge strains circulating in the field and, importantly, to reduce viral shedding, as Dewidar *et al.* (2022) and Sultan *et al.* (2021) stated that vaccine-matched antigen is more protective than the non-vaccine matched one. Group 1: Viral shedding was detected on the 5^{th} day post-challenge (DPC) with moderate CT values and viral concentrations, indicating some level of protection as the virus was still detectable. However, no virus was detected by the 3^{rd} or 7^{th} DPC, suggesting effective initial control and eventual clearance of the virus. This agreed with Ghanem *et al.* (2023), who demonstrated that the HVP360 vaccine showed lesser degrees of viral shedding than the infective dose, which means good control of viral shedding. Group 2: The virus was detected only once with a very low titer at the tested DPCs, indicating strong protection against NDV, which confirms the control of virus shedding (Dewidar *et al.,* 2022), and also confirms the results of Sultan *et al.* (2024) that HVT310 vaccines showed significantly lower virus shedding levels. Group 3: Viral shedding was detected on the 5^{th} and 7^{th} days post-challenge (DPC), with moderate CT values and viral concentrations on both days, which means moderate protection. For group 5, no cloacal viral shedding was detected on any day of the experiment, indicating that none of the groups were exposed to a field infection.

The results suggest that group 2 provided the most effective control of NDV, with low virus detected. However, group 1 showed moderate protection, with some virus shedding detected on the 5^{th} DPC but not on the 3rd or 7^{th} DPC, indicating a temporary increase in viral load that was eventually cleared. The results indicate that viral shedding was minimal or undetectable in the vaccinated groups at all time points measured, suggesting a potential reduction in virus transmission following vaccination. The result of the one-way ANOVA shows an F-value of approximately 4.40 and a P-value of approximately 0.067. Since the P-value is slightly above the commonly used significance threshold of 0.05, this suggests that there is no statistically significant difference in the EID50/ml values between Group 1, Group 3, and the positive control at the 5% significance level. Group 1: Virus shedding was observed only on the 5^{th} DPC, with CT values between 29.16 and 32.08. Viral concentrations ranged from 3.267×10^3 to 2.419×10^4 EID_{50}/ml.

The inclusion of an additional vaccine at 15 days of age provided further protection against NDV. Birds in groups vaccinated with this additional vaccine exhibited decreased viral shedding and reduced clinical symptoms upon challenge. This live vaccine, composed of the ND strain Clone, has been shown to induce strong immune responses, contributing to the observed decrease in virus transmission and shedding. Previous studies have demonstrated that this vaccine effectively reduces NDV shedding, thereby minimizing virus spread within poultry populations (Kapczynski *et al.,* 2013).

The study result aligned with Sultan *et al.*(2024) that HVT-vectored vaccines are effective in controlling viral shedding and providing robust protection against NDV challenges. Combining these vaccines with additional strategies can further enhance protection and minimize virus transmission in poultry populations.

The study underscores the significance of vaccine regimens in managing NDV infection and minimizing viral shedding. Sedeik *et al.* (2022) highlighted that combining different types of vaccines and employing prime-boost strategies can significantly enhance protection and reduce virus transmission. Our findings confirm the effectiveness of genotype-matched and vectored vaccines and the added benefit of live vaccines such as Colone in controlling NDV outbreaks and reducing viral shedding.

In the context of our research on the efficacy of various Newcastle Disease (ND) vaccines, it is pertinent to consider the findings of van Hulten *et al.* (2021), who developed a double construct HVT vaccine (HVT-ND-IBD) incorporating the Newcastle disease virus (NDV) F gene and the infectious bursal disease virus (IBDV) VP2 gene. This vaccine, administered either subcutaneously at 1 day of age or via *in-ovo* vaccination, demonstrated significant protection against multiple strains of velogenic NDV. Notably, early immunity against Newcastle disease was achieved as soon as 2 weeks post-vaccination. Moreover, long-term efficacy was confirmed with NDV protection lasting up to 60 weeks.

Our findings complement those of van Hulten *et al.*(2021), as both studies highlight the advantages of using recombinant and double-vector vaccines for enhanced poultry disease control. While van Hulten *et al.* focused on multi-disease protection and long-term efficacy, our research emphasizes specific protective outcomes and the comparative efficacy of different vaccines against Newcastle Disease. Also, our study provided that the combination of live NDV vaccine and rHVT NDV vector vaccine with each other was able to provide the birds with full protection against mortality.

The serological result p-values indicate the statistical significance of differences in mean antibody titers between study groups at various time points, indicating the impact of the treatments on immune response. Day 14: Significant Differences: The p-value demonstrates notable differences between groups. Group 1 shows a significant increase in mean titer, although not the highest, while Group 3 exhibits the highest mean titer. This indicates that treatments are beginning to show their effects, with some groups, particularly Group 3, producing higher titers. The p-value highlights that these differences are statistically significant. Day 21: Significant Differences: A p-value confirms significant differences, with Group 3 preceding in mean titer, followed by Group 1. By Day 21, the effectiveness of the treatments continues to diverge, with some vaccines achieving higher titers, reflecting growing differences in immune responses among the treatments. Day 28 :Significant Differences: The p-value shows significant differences, with Group 1 and Group 3 both exhibiting high mean titers. Group 3 achieves the highest mean titer, suggesting superior performance at this stage. The substantial rise in titers for both groups indicates strong immune responses induced by these treatments by Day 28. Day 31: Significant Differences: A p-value indicates significant

differences, with Group 1 and Group 3 showing high mean titers, and Group 2 also performing well. By Day 31, treatments are maintaining strong immune responses, with Group 1 and Group 3 demonstrating particularly high titers. Day 35: Significant Differences: The p-value indicates significant differences, with Group 1 reaching its peak mean titer and Group 2 achieving the highest mean titer. The consistently high titers observed for Group 1 suggest effective long-term immunity, while Group 2 shows superior performance on this day.

When comparing Group 1 to the other treatments, it is evident that while it may not always have the highest mean titer on each day, it is consistently one of the top performers, especially at the later stages. This suggests that Group 1 could be a preferred choice for long-term immunity, though its performance should be considered in conjunction with other factors, such as variability and comparison to other treatments like Group 2 and Group 3, and these results agree with van Hulten *et al.* (2021)

The significant p-values (less than 0.05) observed indicate that there are differences between most of the study groups and across different time points. These findings suggest that the treatments (particularly those in Groups 1, 2, and 3) are effective in producing significant changes over time, and this proves the results of Sultan *et al.* (2024) and Ghanem *et al.* (2023).

The consistent statistical significance across different time points underscores the effectiveness of the treatments in generating a strong immune response. While some treatments, like Group 1, may not always have the highest mean titers, they offer robust and sustained immune responses. Conversely, Groups 2 and 3 show high titers at various stages, demonstrating their effectiveness in eliciting strong immune responses. The variability in standard deviations, particularly in the early days, points to differences in individual responses, but by Day 35, the high consistency among the top-performing groups indicates reliable and effective long-term immune protection.

In comparing the efficacy of Newcastle Disease (ND) vaccines, several studies offer valuable insights. Our study evaluated the effectiveness of recombinant vaccines, including vaccines used in groups 1, 2, and 3 in one-day-old SPF chicks. Our findings indicated that all used recombinant vaccines provided effective protection and prevented mortalities compared to the control groups. Conversely, Ghanem *et al*. (2023) assessed the protective effects of (rHVT-NDV-IBDV) vaccines when combined with a live-attenuated NDV vaccine in broiler chicks with maternally derived antibodies. This study demonstrated that combining or boosting these vector vaccines with a live NDV vaccine enhanced immunity, reduced mortality, and minimized virus shedding more effectively than using the vector vaccines alone. Both studies highlight the effectiveness of recombinant and combined vaccination strategies in controlling Newcastle Disease, though they reveal different strengths in vaccine performance and immune response.

Overall, the significant p-values and the observed patterns in mean titers suggest that the treatments are effective in enhancing immune responses.

CONCLUSION

The results of this study demonstrated the critical role of recombinant vaccines, especially those based on the Herpes Virus of Turkey (HVT), in managing Newcastle Disease Virus (NDV) outbreaks in Egypt and similar regions. The effectiveness of both recombinant and live vaccine combinations indicates a promising approach to enhancing herd immunity and reducing viral shedding in poultry populations. This is particularly important in areas where NDV genotype VII strains are prevalent, as these strains are associated with increased virulence and

significant economic losses in the poultry sector. The HVT-vectored vaccines not only reduce mortality but also improve overall immune responses against virulent NDV strains. By adopting advanced vaccination strategies that incorporate HVT-based vaccines alongside conventional live vaccines, poultry producers can lessen the impact of NDV, ensuring better animal health, improving productivity, and contributing to food security in regions that heavily depend on poultry farming. So, we concluded that these strategies not only help to reduce clinical symptoms and mortality rates but also lower the risk of transmission within flocks, promoting more sustainable poultry farming practices in the face of persistent viral threats.

REFERENCES

Abd El-Hamid, H.S. and Soliman, M.A. (2020): The role of recombinant vaccines in controlling Newcastle disease virus outbreaks in Egypt. In Proceedings of the International Conference on Veterinary Medicine, 145-15.

Ahmed, H. M., Amer, M. M., El-bayoumi, K., Amer, S. A., & Kutkat, M. A. E. A. 2017. Identification and sequencing of Genotype VII of Newcastle disease virus from chicken flocks in six Egyptian Governorates. Egyptian Journal of Veterinary Sciences, 48(1), 31-41.

Aini, I. (1990): Newcastle disease vaccines: A review. Avian Pathology 1, 13-32. https://doi.org/10.1080/03079459008418858

Alexander, D.J. (2000): Newcastle disease and other avian paramyxoviruses. *Revue Scientifique et Technique-Office International des Epizooties, 19* 2, 443-455\. In A. M. Saif (Ed.), Diseases of poultry 541-57. Iowa State University Press.

Amin, M.A.; Ghanem, M. and Younis, M. (2022): Newcastle disease in poultry: Clinical manifestations and control strategies. Poultry Science 12, 100-109. https://doi.org/10.1016/j.psj.2022.100123

Bancroft, J.D. and Gamble, M.N. (2008): Theory and practice of histological techniques 6th ed. Churchill Livingstone.

Chumbe, A., Izquierdo-Lara, R., Calderón, K., Fernández-Díaz, M. and Vakharia, V.N., 2017. Development of a novel Newcastle disease virus (NDV) neutralization test based on recombinant NDV expressing enhanced green fluorescent protein. Virology journal, 14.1-11.

Dewidar, A.A.A.; Kilany, W.H.; El-Sawah, A.A.; Shany, S.A.S.; Dahshan, A.-H.M.; Hisham, I.; Elkady, M.F.; Ali, A. (2022): Genotype VII.1.1-Based Newcastle Disease Virus Vaccines Afford Better Protection against Field Isolates in Commercial Broiler Chickens. *Animals* 2022, *12*, 1696. https://doi.org/10.3390/ani12131696

Dortmans, J.C.F.M.; Koch, G. and Peeters, B. (2011): Newcastle disease virus vaccines: An overview of the current and potential future vaccines. Veterinary Microbiology1-2, 1-8. https://doi.org/10.1016/j.vetmic.2011.05.026

El-Sayed, A. and Eldeeb, A. (2021): Recombinant HVT vaccines as an effective strategy for controlling Newcastle Disease in poultry. Avian Diseases 4, 524-534. https://doi.org/10.1637/avdi-2021-0321

El-Sayed, N.; El-Sabry, M. and Abouelmagd, A. (2023): Evaluation of INNOVAX-ND-IBD® vaccine in controlling Newcastle Disease and Infectious Bursal Disease in poultry. Journal of Veterinary Science and Technology 2, 59-68. https://doi.org/10.4172/2157-7579.1000534

Fadly, A.M. and Hatta, M. (2019): Advances in Newcastle disease vaccines: a review. Journal of Veterinary Medicine and Animal Health 1, 1-11. https://doi.org/10.5897/JVMAH2018.0674

Fawzy, M., Ali, R.R., Elfeil, W.K., Saleh, A.A. and El-Tarabilli, M.M.A., 2020. Efficacy of inactivated velogenic Newcastle disease virus genotype VII vaccine in broiler chickens. In Veterinary Research Forum (Vol. 11, No. 2, p. 113).

Gergen, L.; Machek, A. and van Hulten, M.C.W. (2019): A double recombinant herpes virus of turkeys for the protection of chickens against Newcastle, infectious laryngotracheitis, and Marek's diseases. Avian Pathology 1, 45-56. https://doi.org/10. 1080/ 03079457.2018.1485022

Ghanem, I.A.E.; Abdullatif, T.M. and Hassanin, O. (2023): The protection conferred against virulent Newcastle Disease Virus Genotype VII by commercial double recombinant HVT vaccines and NDV live-attenuated vaccine as prime/boost vaccination regimens in commercial broiler chickens. Avian Pathology 4, 251-263. https://doi.org/10.1080/03079457.2023 .2211548

Hassan, M.K.; Youssef, S.M. and Maher, S.S. (2009): Evaluation of Nobilis® ND Clone 30 vaccine in controlling Newcastle disease. Veterinary Record 6, 174-176. https://doi.org/10.1136/vr. 164.6.174

Hussein, E.A.; Hair-Bejo, M.; Adamu, L.; Omar, A.R. and Arshad, S.S. (2018): Scoring system for lesions induced by different strains of Newcastle disease virus in chickens. Veterinary Medicine International, 2018, 1-9. https://doi.org/ 10.1155/2018/8674526

Hussein, R.M.; Fathy, H. and El-Shafei, R. (2018): Histopathological changes in the brain of chickens infected with Newcastle disease virus. Avian Diseases 2, 152-160. https://doi.org/10. 1637/11854-111517-Reg.1

Jia, W.; Chen, X.; Yu, Y.; Wu, Q.; Zhu, X. and Wang, H. (2022): Construction and immune efficacy of a recombinant turkey herpesvirus vaccine strain expressing a fusion protein of genotype VII Newcastle disease virus. Veterinary Microbiology, 268, 109429. https://doi.org/10.1016/j.vetmic.2022.1 09429

Kapczynski, D.R.; Afonso, C.L. and Miller, P.J. (2013): Vaccine technologies for Newcastle disease virus: Focus on genetically engineered and vector vaccines. Poultry Science 5, 1349-1358. https://doi.org/10.3382/ps.2012-02670

Lee, J.; Lee, C-W.; Suarez, DL.; Lee, SA.; Kim, T. and Spackman, E (2024): Efficacy of commercial recombinant HVT vaccines against a North American clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus in chickens. PLoS ONE 7: e0307100. https://doi.org/10.1371/journal.pone .0307100

Mayo MA. 2002. A summary of taxonomic changes recently approved by ICTV. Archive virol.,147 (8):1070-1076.

Miller, P.J. and Koch, G., 2013. Newcastle disease. Diseases of poultry, 13, pp.89-138.

Moharam, I.; Razik, A.A.el.; Sultan, H.; Ghezlan, M.; Meseko, C.; Franzke, K. 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 Pathology 5, 406–415. https://doi.org /10.1080/ 03079457.2019.1612852

Mousa, M.R.; Mohammed, F.F.; Abdel Raheem, F.A.; El-Deeb, A.H. and Ahmed, K.A. (2020): Characterization of renal inflammatory cytokines and related nephropathy in experimentally infected broiler chickens with velogenic Newcastle disease virus. Journal of World Poultry Research 1, 109-117. https://doi.org/10.36380/ jwpr.2020.15

Mousa, M.R.; Mohammed, F.F.; El-Deeb, A.H.; Amer, F. and Ahmed, K.A. (2019): Comparative serological, histopathological, and

immunohistochemical evaluation of the immune status of broiler chickens experimentally infected with velogenic Newcastle disease virus at different ages. International Journal of Veterinary Science 3, 143-150. https://doi.org/10.17582/journal.ijvs/2019/8.3.143.150

Mousa, M.R.; Mohammed, F.F.; El-Deeb, A.H.; Amer, F. and Ahmed, K.A. (2021): Pathological monitoring of visceral lesions induced by velogenic Newcastle disease in experimentally infected broiler chickens at different ages with special reference to pulmonary inflammatory cytokines expression. Exploratory Animal Medical Research1, 67-79. https://doi.org/10.52635/EAMR/11.1.67-79

Murulitharan, K.; Yusoff, K.; Omar, A.R. and Molouki, A. (2013): Characterization of Malaysian velogenic NDV strain AF2240-I genomic sequence: A comparative study. Virus Genes, 46, 431-440. https://doi.org/10.1007/s11262-013-0896-3

Naeem, M.; Alkhodairy, HF.; Ashraf, I. Khalil, AB. (2022): CRISPR/Cas System Toward the Development of Next-Generation Recombinant Vaccines: Current Scenario and Future Prospects. Arab J Sci Eng. 2023;48(1):1-11. doi: 10.1007/s13369-022-07266-7. Epub 2022 Sep 23. PMID: 36185592; PMCID: PMC9510529.

OIE 2012. Newcastle disease. In Terrestrial Animal Health Code (228-240). World Organization for Animal Health.

Sultan, H.A.; Tantawi, L. and Gad, T. (2024): The protective efficacy of recombinant Herpesvirus of Turkey (rHVT-ND-IBD) vaccine against velogenic Newcastle Disease Virus Genotype VII. Journal of Current Veterinary Research 1, 161-185. https://doi.org/10.1016/j.jcvr.2024.01.002

Sultan, H.A.; Elfeil, W.K.; Nour, A.A.; Tantawy, L.; Kamel, E.G.; Eed, E.M. and Talaat, S. (2021): Efficacy of the Newcastle disease virus genotype VII. 1.1-matched vaccines in commercial broilers. *Vaccines*, *10*(1), 29. Tizard, I. R., 2004. Veterinary immunology: An introduction (8th ed.). https://doi.org/10.3390/vaccines10010029

Saunders van Hulten, M.C.W.; Cruz-Coy, J.; Gergen, L.; Pouwels, H.; Ten Dam, G.B.; Verstegen, I.; De Groof, A.; Morsey, M. and Tarpey, I. (2021): Efficacy of a turkey herpesvirus double construct vaccine (HVT-ND-IBD) against challenges with different strains of Newcastle disease, infectious bursal disease, and Marek's disease viruses. Avian Pathology1, 18–30. https://doi.org/10.1080/03079457.2020.1828567

Wise, M.G.; Suarez, D.L.; Seal, B.S., Pedersen, J.C.; Senne, D.A.; King, D.J. and Spackman, E. (2004): Development of a real-time reverse-transcription PCR for the detection of Newcastle disease virus RNA in clinical samples. Journal of Clinical Microbiology 7, 329-336. https://doi.org/10.1128/JCM.42.7.329-336.2004

فعالية اللقاحات المؤتلفة القائمة على فيروس هربس الديك الرومي (HVT) في نمطيها الأحادي والثنائي مع جرعة تعزيزية بلقاح نيوكاسل الحي ضد فيروس نيوكاسل شديد الضراوة من النمط الجينيVII السابع

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تُقيّم هذه الدراسة فعالية تركيبات لقاح نيوكاسل المؤتلف في كتاكيت خالية من مسببات الأمراض النوعية (SPF) بعمر يوم واحد، مع التركيز على أدائها ضد سلالة فيروس نيوكاسل (Egypt-NDV-RLQP-2021) المنتشرة حاليًا في مصر.
تم تقسيم ١٠٠ كتكوت SPF إلى خمس مجموعات:
المجموعة ١ :تلقت لقاح.®INNOVAX-ND-IBD
المجموعة ٢ :تم إعطاؤها لقاح.®VAXXITEK
المجموعة ٣ :تلقتVectormune® ND ، الذي يستخدم (HVT)ناقل من خلال إدماج الجين *F*من فيروس نيوكاسل في جينوم.HVT
تم تطعيم المجموعات بلقاح حي من النمط الجيني الثاني من فيروس نيوكاسل (NDV genotype II) عند عمر ١٥ يومًا بطريقة التقطير داخل العين.
المجموعة ٤ :كانت المجموعة الضابطة الإيجابية (لم تتلقَ تطعيمًا وتم تعريضها للفيروس)
المجموعة ٥ :كانت المجموعة الضابطة السلبية (لم تتلقَ تطعيمًا ولم تتعرض للفيروس)
بعد ٢٨ يومًا، تم تعريض المجموعات (١، ٢، ٣، ٤) لفيروس نيوكاسل شديد الضراوة من النمط الجيني السابع بجرعة ٠,٢ مل تحتوي على {50}_EID50 6^10610من الفيروس بطريقة الحقن العضلي.
تمت مراقبة الكتاكيت لرصد الأعراض السريرية، معدل الوفيات، ومستوى الفيروس باستخدام تقنية qPCR في الوقت الحقيقي. كما أُجريت تقييمات نسيجية مرضية والرئتين والدماغ واللوز الأعورية باستخدام صبغة H&E.
النتائج:
أظهرت النتائج النسيجية المرضية أن جميع المجموعات التي تلقت التطعيم قدمت تحكمًا جيدًا في الحد من التلف النسيجي مقارنة بالمجموعة ٤.
الفحوصات المصلية أكدت وجود زيادة معنوية في مستويات الأجسام المضادة بعد التطعيم والتحدي في جميع المجموعات المطعمة، مما يشير إلى توفير مناعة واقية ضد الفيروس.
فيما يتعلق بطرح الفيروس، أظهرت المجموعة ٢ عدم وجود الفيروس إطلاقًا في أي وقت، مما يشير إلى سيطرة فائقة على طرح الفيروس.
الاستنتاج:
توصي الدراسة بشدة باستخدام اللقاحات القائمة على HVT مع لقاحات النيو كاسل الحية، حيث قدمت حماية سريرية كاملة ضد النمط الجيني السابع من فيروس نيوكاسل.