Comparative Efficacy of Genotype-Matched and Conventional Vaccines Against Newcastle Disease Virus Genotype VII in Broilers

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Abstract:

Background:

Newcastle disease (ND) is one of the most economically significant diseases in the poultry industry. In Iran, the disease is endemic and frequently causes outbreaks with high mortality and severe clinical signs, predominantly associated with genotype VII strains. The poultry industry in Iran is essential for food security and economic stability; however, viral diseases, particularly Newcastle disease, pose a major threat by causing significant production losses and economic damage. The aim of this study was to evaluate the serological and performance efficacy of the MEVAC RINNOVAC Eli-7 and MEVAC ND7 PLUS vaccines.

Methods:

A total of 150 commercial LSL layer cockerels were divided into six groups and maintained under identical conditions. Birds were vaccinated at 14 days of age with live or inactivated NDV vaccines. Blood samples were collected on days 0, 14, and 21 post-vaccination to evaluate serological responses using the HI test. On day 21 post-vaccination, birds were challenged with a circulating NDV genotype VII strain, and clinical signs, viral shedding (via RT-PCR), and pathological changes were monitored. Molecular and phylogenetic analyses of the fusion (F) gene were performed to confirm vaccine strain identity.

Results**:**

Both MEVAC ND7 PLUS and MEVAC RINNOVAC Eli-7 vaccines elicited robust serological responses by day 21 post-vaccination, with antibody titers significantly higher than those of the unvaccinated control and comparable to conventional vaccines. Following the challenge on day 28, vaccinated birds maintained high antibody levels and showed no clinical or histopathological lesions, whereas control birds did not survive. Vaccine uptake assessed by RT-PCR on day 5 post-vaccination was undetectable in all groups.

Conclusion:

Both MEVAC RINNOVAC Eli-7 and ND7 PLUS vaccines effectively induced protective immunity, reduced viral replication and shedding, and elicited robust antibody responses against NDV genotype VII. These results underscore the value of genotype-matched vaccination as a key strategy for controlling Newcastle disease and minimizing its economic impact in poultry.

Key words: MEVAC RINNOVAC Eli-7, ND7 PLUS, Newcastle disease virus, vaccine

Introduction

As a key component of agricultural systems, the poultry sector contributes substantially to animal protein supply, food security, and job creation in many regions of the world, including Iran [1]. The poultry industry is persistently challenged by multiple risk factors, including nutritional imbalances, infectious diseases, suboptimal vaccination strategies, management deficiencies, and inadequate biosecurity practices. Among these challenges, viral diseases are of particular importance due to their substantial contribution to increased mortality rates, impaired growth performance, and significant reductions in production output [2]. Among viral diseases, Newcastle

disease remains one of the most important threats to the poultry industry, as despite decades of vaccination, it continues to cause significant economic losses worldwide [3]. *Newcastle disease virus* (NDV), the most important pathogen among the Orthoavulaviruses, is a highly contagious virus affecting domestic poultry and a wide range of wild birds. NDV is classified as a strain of *Avian paramyxovirus 1* (APMV-1, species *Avian orthoavulavirus 1*) and belongs to the family *Paramyxoviridae*, genus *Avulavirus*. It is an enveloped, negative-sense, single-stranded RNA virus with a genome encoding six structural proteins (N, P, M, F, HN, and L). The F protein cleavage site plays a key role in determining its virulence. Virulent NDV strains are endemic in poultry in much of Asia, Africa, and parts of the Americas, while countries like the USA and Canada remain free through import restrictions and culling of infected birds. Low-virulence NDV strains are widespread in poultry and wild birds, with chickens being the most susceptible domestic species, including pigeon-adapted variants of APMV-1 [4]. Newcastle disease virus (NDV) continues to evolve, resulting in the classification of approximately twenty genotypes based primarily on genetic variation in virulence-associated genes, particularly the fusion (F) gene. The F protein plays a central role in viral pathogenicity, and its amino acid cleavage site is considered the major determinant of NDV virulence. Highly virulent strains are capable of causing systemic infection and severe lesions in chickens. This genetic diversity has been associated with the occurrence of disease outbreaks even in vaccinated poultry flocks [5]. Although most currently used ND vaccines are derived from early genotypes (I and II), the recent emergence and widespread circulation of highly virulent NDV strains, particularly genotype VII (GVII), which are genetically and antigenically distinct from traditional vaccines, have increased disease severity and complicated control strategies in poultry production systems [6, 7]. Despite the extensive use of vaccination programs, Newcastle disease (ND) outbreaks continue to occur frequently in Iran. Previous molecular studies based on partial or complete sequencing of the fusion (F) and hemagglutinin–neuraminidase (HN) genes have confirmed the circulation of NDV genotype VII in Iranian poultry populations. Recent outbreaks characterized by high mortality rates and severe clinical manifestations further highlight the ongoing prevalence and epidemiological importance of genotype VII NDV in Iran [8]. Reports of vaccine failure in controlling Newcastle disease outbreaks have raised concerns regarding the limited ability of conventional vaccines to reduce viral replication and shedding effectively. Consequently, increasing attention has been directed toward the development of genotype-matched vaccines homologous to circulating virulent NDV

strains [9]. Evidence indicates that higher levels of humoral immunity in vaccinated birds are associated with reduced infection rates, viral replication, and shedding, an effect more readily achieved with homologous vaccines; however, sufficiently high heterologous antibody titers, particularly induced by oil-adjuvanted inactivated vaccines, can also effectively suppress virulent NDV shedding, supporting their use even in broiler chickens [10]. To date, a wide range of live attenuated and inactivated Newcastle disease vaccines have been extensively used in poultry production systems, primarily to mitigate clinical disease and reduce mortality. Nevertheless, these conventional vaccines are largely derived from early NDV genotypes and may be suboptimal in limiting viral replication and shedding of currently circulating virulent strains. In recent years, reverse genetics–based vaccines have emerged as a promising next-generation approach, enabling precise genetic manipulation, improved safety profiles, and enhanced antigenic matching with field viruses [11, 12]. Accordingly, to the best of our knowledge, the present study represents the first evaluation in Iran of a reverse genetics-derived live NDV vaccine (MEVAC RINNOVAC Eli-7) against circulating genotype VII strains under experimental conditions.

Materials and Methods

Experimental Design

To minimize potential bias, all factors that could influence the experimental outcomes were standardized across groups, and birds were maintained in separate cages under identical conditions throughout the study. A total of 150 commercial LSL layer cockerels were obtained from Barekat Poultry Company and randomly allocated into six experimental groups at the animal facility of the Faculty of Veterinary Medicine. All groups received a standardized diet. Baseline blood samples were collected from the control group on day 0 and assessed using the hemagglutination inhibition (HI) test.

Vaccination Protocol

Vaccination Protocol and Experimental Groups. The study evaluated two primary commercial genotype-matched vaccines: MEVAC RINNOVAC Eli-7, a reverse genetics-derived live attenuated lyophilized recombinant vaccine containing the rNDV: VG/GA-F7 strain (titer $\geq 10^{6.5}$ EID_{50} per dose), and MEVAC ND7 PLUS, an inactivated oil-adjuvanted recombinant vaccine containing both Genotype VII and LaSota strains ($\leq 10^{8.5}$ EID_{50} of each per dose). For comparison,

conventional vaccines were utilized, including a standard lyophilized live vaccine (Live A) and an oil-emulsion inactivated vaccine (Inactive Conventional). Two control groups were maintained as placebos: one group remained unvaccinated but was later challenged to assess morbidity and mortality (Positive Control), while the second remained both unvaccinated and unchallenged to serve as a negative control for baseline serological and pathological parameters (Negative Control). All vaccinations were administered at 14 days of age. Live vaccines were reconstituted in their respective diluents and administered, while inactivated vaccines were administered via injection after reaching room temperature. Final vaccine intake was verified 5 days post-vaccination through RT-PCR analysis of tracheal and oral swabs in the live vaccine groups.

Serological and pathological evaluation:

Lyophilized MEVAC RINNOVAC Eli-7 and the conventional live vaccine (Live A) were reconstituted in their respective diluents, mixed, and diluted appropriately before administration as detailed in Table 1. Similarly, the inactivated MEVAC ND7 PLUS vaccine and a conventional inactivated vaccine were thoroughly mixed at room temperature and administered to the birds as specified in Table 1. Five days post-vaccination, five birds from each vaccinated group were euthanized, and tracheal samples were collected for pathological examination. Serological responses were evaluated by hemagglutination inhibition (HI) testing, using 4 HA units of the La Sota antigen, on days 0, 14, and 21 post-vaccination, with day 21 testing using both commercial and circulating genotype VII antigens.

On day 21 post-vaccination (at 35 days of age), birds were challenged with a virulent circulating Newcastle Disease Virus (NDV) Genotype VII strain isolated by the Virology Laboratory at the University of Tehran. To ensure a robust infection that mimics field exposure, each bird received a challenge dose of $10^{6.0}$ EID_{50}. The challenge was administered via the intraocular and intranasal routes (0.1 ml total volume, 0.05 ml per site), a standard method for inducing both mucosal and systemic immune responses in vaccine efficacy trials. Five days post-challenge, oral and cloacal swabs were collected for RT-PCR analysis, and five birds from each group were euthanized for tracheal pathology assessment. Clinical signs were monitored throughout the study. Finally, on day 28 post-vaccination (7 days post-challenge), blood samples were collected from the remaining birds for HI evaluation.

Vaccine uptake assessment:

To evaluate vaccine uptake, oral and cloacal swabs were collected on day 5 post-vaccination from birds in the groups receiving live vaccines, as indicated in Table 1. Samples were analyzed using RT-PCR and real-time PCR with NDV-specific primers.

Genetic and phylogenetic evaluation of the vaccine:

For genetic and phylogenetic evaluation of the MEVAC RINNOVAC Eli-7 vaccine, RNA and DNA were extracted from the live vaccine, and the strain was confirmed molecularly and phylogenetically based on the fusion (F) gene. The vaccine was also examined for external microbial and viral contaminants, including adenovirus, Mycoplasma, infectious bronchitis virus, and avian influenza virus. Vaccine seed RNA and DNA from Rooyan Darou Company were transported on ice to the Virology Laboratory of the Faculty of Veterinary Medicine, University of Tehran, and stored at −20 °C until cDNA synthesis.

cDNA synthesis:

cDNA was synthesized using the RevertAid H Minus First Strand cDNA Synthesis Kit based on the manual of the kit.

PCR for Newcastle disease Fusion (F) gene amplification:

PCR amplification of the Newcastle disease Fusion (F) gene was performed under the following conditions: initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 15 s, annealing at 48 °C for 30 s, and extension at 68 °C for 30 s, repeated for 34 cycles, followed by final extension at 68 °C for 7 min. PCR products were analyzed on 1.5% agarose gel, and the expected band length of the Fusion gene was 203 bp.

PCR for detecting infectious bronchitis, avian influenza, adenovirus, and Mycoplasma:

Diagnostic RT-PCR and PCR assays were performed to detect infectious bronchitis virus, avian influenza virus, adenovirus, and Mycoplasma, all of which were negative. Positive RT-PCR samples, together with the Fusion gene primers, were sent on ice for one-directional sequencing to Codon Genetics Company.

Phylogenetic and Sequence Analysis of NDV Vaccine Strains

After obtaining the sequences, their reading quality was evaluated using Finch TV software. To determine the genotype and subgenotype of the live vaccine virus MEVAC RINNOVAC Eli-7, the lyophilized vaccine was reconstituted, followed by RNA extraction and cDNA synthesis. PCR amplification was then performed targeting a partial region of the fusion (F) gene containing the cleavage site. The positive RT-PCR product was sequenced, and sequence quality was assessed using FinchTV software. Initial confirmation of sequence identity was performed using the BLAST tool in the NCBI GenBank database. The obtained sequences were aligned with NDV genotype VII reference strains and previously reported Iranian isolates retrieved from GenBank using MEGA7 software. Multiple sequence alignments were generated, and phylogenetic analysis was conducted using the Maximum Likelihood (ML) method based on the General Time Reversible (GTR) model, with 1000 bootstrap replicates used to assess tree robustness.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism software. Differences in hemagglutination inhibition (HI) antibody titers, viral shedding, and other continuous variables among the four groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Survival rates were analyzed using Kaplan–Meier survival analysis and compared with the log-rank (Mantel–Cox) test. Clinical scores and growth performance data were also assessed using appropriate parametric or non-parametric tests, as applicable, to ensure robust evaluation of vaccine efficacy.

Table 1. Experimental grouping, vaccination protocol, serological sampling schedule, vaccine uptake assessment, and challenge design.

Group	Number of Chickens	Vaccine Type	Vaccination at Day 14	Vaccine Uptake (Day 5)	HI Test (Day 14)	HI Test (Day 21)	Challenge & Sampling
Live Vaccines	25	MEVAC RINNOVAC Eli-7	+	+	+	+	+
	25	Conventional Live	+	+	+	+	+
Inactive Vaccines	25	MEVAC ND7 PLUS	+		+	+	+

	25	Conventional Inactive	+		+	+	+
Controls	25	Positive Control	Unvaccinated (Placebo)		+	+	+
	25	Negative Control	Unvaccinated (Placebo)		+	+	-

193

194 **Results:**

195 **Serological evaluation of the MEVAC ND7 PLUS vaccine 14 days post-vaccination using a**
196 **commercial antigen:**

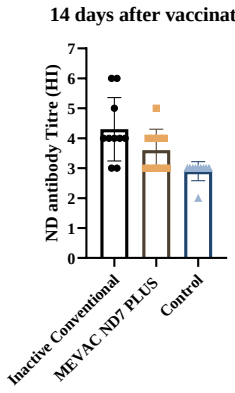
197 Statistical analysis between the MEVAC ND7 PLUS group (3.60 ± 0.699) and the Inactive
198 conventional vaccine group (4.30 ± 1.059) showed no significant difference ($P > 0.1146$).
199 Comparison of antibody titers between the MEVAC ND7 PLUS group (3.60 ± 0.699) and the
200 control groups (2.22 ± 0.421) also showed no significant difference ($P > 0.1146$), whereas a
201 significant difference was observed between control groups (2.22 ± 0.421) and the Inactivated
202 conventional vaccine group (4.30 ± 1.059) ($P < 0.0009$) (Figure 1(a)).

203 **Serological evaluation of the MEVAC RINNOVAC Eli-7 vaccine 14 days post-vaccination**
204 **using a commercial antigen:**

205 On day 14 post-vaccination, blood samples were collected from chicks in each group to evaluate
206 Newcastle antibody titers. Comparison of antibody titers between the MEVAC RINNOVAC Eli-7
207 group (3.60 ± 0.516) and the Live A vaccine group (4.100 ± 0.7379) showed no significant
208 difference ($P > 0.1246$). Moreover, a comparison of antibody titers between the control groups
209 (2.90±0.316) and all vaccine-receiving groups showed a significant difference ($P < 0.0005$) (Figure
210 1(b)).

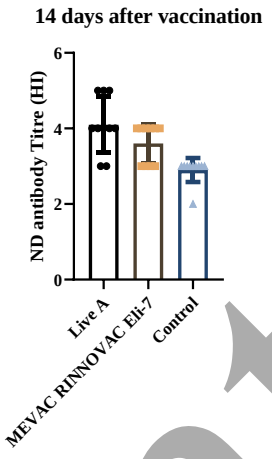
211

(a)



	Inactive Conventional	MEVAC ND7 PLUS	Control
Mean	4.300	3.600	2.900
Std. Deviation	1.059	0.6992	0.3162
Std. Error of Mean	0.3350	0.2211	0.1000

(b)



	Live A	MEVAC RINNOVAC Eli-7	Control
Mean	4.100	3.600	2.900
Std. Deviation	0.7379	0.5164	0.3162
Std. Error of Mean	0.2333	0.1633	0.1000

Figure 1. (a) Serological evaluation of the MEVAC ND7 PLUS vaccine 14 days post-vaccination. Comparison of antibody titers against Newcastle vaccines and the control groups using a commercial antigen. (b) Evaluation of Newcastle antibody titers 14 days post-vaccination with MEVAC RINNOVAC Eli-7. Comparison of antibody titers against Newcastle vaccines and the control groups using a commercial antigen.

Serological evaluation of the MEVAC ND7 PLUS vaccine 21 days post-vaccination using a commercial antigen:

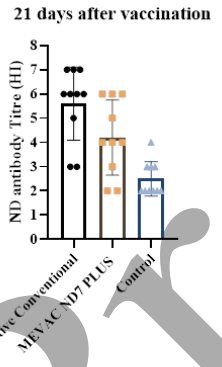
Comparison of Newcastle antibody titers induced by the MEVAC ND7 PLUS vaccine (4.20 ± 1.549) and the Inactive conventional vaccine group (5.6 ± 1.506) showed no significant difference ($P > 0.0611$). In the evaluation of antibody titers, the control groups (2.5±0.707) showed a significant difference compared to both vaccine-receiving groups ($P < 0.0005$) (Figure 2(a)).

Serological evaluation of the MEVAC RINNOVAC Eli-7 vaccine 21 days post-vaccination using a commercial antigen:

On day 21 post-vaccination, blood samples were collected from chicks in each group to evaluate Newcastle antibody titers. Comparison of antibody titers between the MEVAC RINNOVAC Eli-7

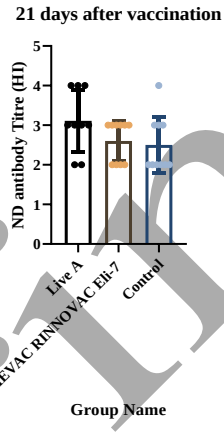
group (2.60 ± 0.516) and the Live A vaccine group (2.5 ± 0.7071) showed no significant difference (P > 0.2426). Furthermore, comparison of antibody titers between the control groups (2.50±0.707) and the MEVAC RINNOVAC Eli-7 group (2.60 ± 0.516) showed no significant difference (P > 0.9557), whereas a significant difference was observed between the control groups (2.50±0.707) and the Live A vaccine group (3.889 ± 1.054) (P < 0.0018) (Figure 2(b)).

(a)



	Inactive Conventional	MEVAC ND7 PLUS	Control
Mean	5.600	4.200	2.500
Std. Deviation	1.506	1.549	0.7071
Std. Error of Mean	0.4761	0.4899	0.2236

(b)

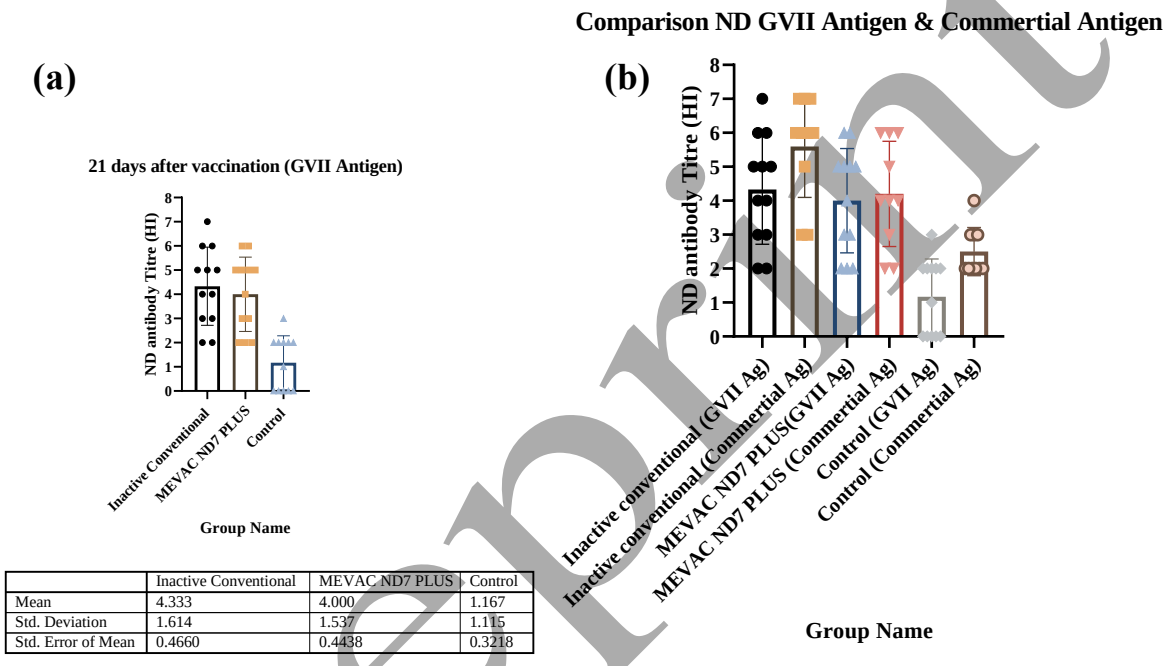


	Live A	MEVAC RINNOVAC Eli-7	Control
Mean	3.111	2.600	2.500
Std. Deviation	0.7817	0.5164	0.7071
Std. Error of Mean	0.2606	0.1633	0.2236

Figure 2. (a) Comparison of serological evaluation of the MEVAC ND7 PLUS vaccine 21 days post-vaccination. Comparison of antibody titers against Newcastle vaccines and the control groups using a commercial antigen. (b) Evaluation of Newcastle antibody titers 21 days post-vaccination with MEVAC RINNOVAC Eli-7. Comparison of antibody titers against Newcastle vaccines and the control groups using a commercial antigen.

Serological evaluation of the MEVAC ND7 PLUS vaccine 21 days post-vaccination using circulating GVII antigen:

Antibody titers induced by the MEVAC ND7 PLUS vaccine (4.00 ± 1.537) and the inactivated conventional vaccine group (4.33 ± 1.614) did not differ significantly (P > 0.8383). Comparison of antibody titers across all groups with the control group (1.167 ± 1.115) revealed a significant difference between all vaccinated groups and the control (P < 0.0001) (Figure 3(a)). Antibody titers of the groups against the two commercial antigens and the circulating GVII antigen are presented in Figure 3(b).



	Inactive Conventional	MEVAC ND7 PLUS	Control
Mean	4.333	4.000	1.167
Std. Deviation	1.614	1.537	1.115
Std. Error of Mean	0.4660	0.4438	0.3218

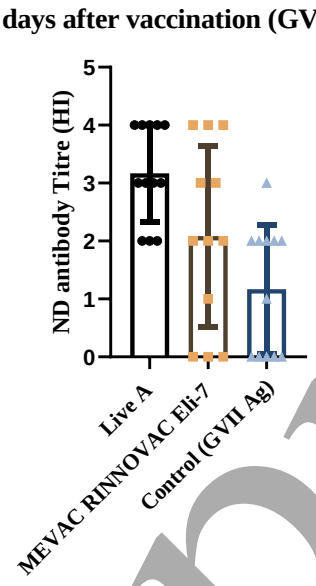
Figure 3(a). Comparison of serological evaluation of the MEVAC ND7 PLUS vaccine 21 days post-vaccination. Comparison of antibody titers against Newcastle vaccines and the control group using circulating GVII antigen. (b) Comparison of serological evaluation of the MEVAC ND7 PLUS vaccine 21 days post-vaccination. Comparison of antibody titers against Newcastle vaccines and the control groups using circulating GVII antigen and commercial antigen.

Serological evaluation of the MEVAC RINNOVAC Eli-7 vaccine 21 days post-vaccination using circulating GVII antigen:

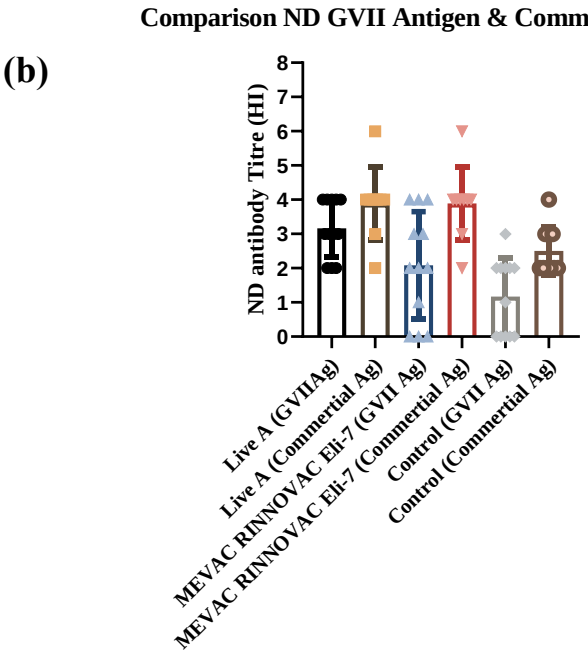
On day 21 post-vaccination, blood samples were collected from chicks in each group to assess antibody titers against Newcastle disease virus. Antibody titers in the MEVAC RINNOVAC Eli-7 group (2.083 ± 1.564) and the Live A vaccine group (3.167 ± 0.8348) did not show a significant difference (P > 0.0870). Statistical analysis revealed a significant difference in antibody titers

between the control groups (1.167 ± 1.115) and all vaccinated groups (P < 0.0008). Comparison between the MEVAC RINNOVAC Eli-7 group (2.083 ± 1.584) and the control groups (1.115 ± 1.167) did not indicate a significant difference (P < 0.1673) (Figure 4(a)). Antibody titers of the groups against the two commercial antigens and the circulating GVII antigen are presented in Figure 4(b).

(a)



	Live A	MEVAC RINNOVAC Eli-7	Control (GVII Ag)
Mean	3.167	2.083	1.167
Std. Deviation	0.8348	1.564	1.115
Std. Error of Mean	0.2410	0.4516	0.3218



	Live A (GVIIAg)	Live A (Commertial Ag)	MEVAC RINNOVAC Eli-7 (GVII Ag)	MEVAC RINNOVAC Eli-7 (Commertial Ag)	Control (GVII Ag)	Control (Commertial Ag)
Mean	3.167	3.889	2.083	3.889	1.167	2.500
Std. Deviation	0.8348	1.054	1.564	1.054	1.115	0.7071
Std. Error of Mean	0.2410	0.3514	0.4516	0.3514	0.3218	0.2236

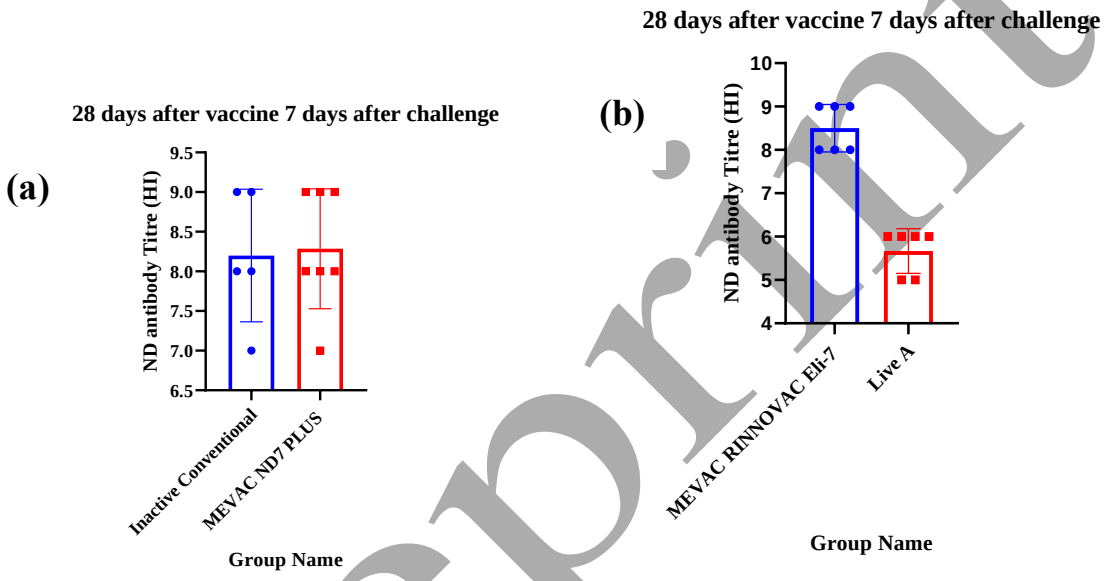
Figure 4 (a). Evaluation of Newcastle antibody titers 21 days post-vaccination with MEVAC RINNOVAC Eli-7. Comparison of antibody titers against Newcastle vaccines and the control groups using circulating GVII antigen. (b) Evaluation of Newcastle antibody titers 21 days post-vaccination with MEVAC RINNOVAC Eli-7. Comparison of antibody titers against Newcastle vaccines and the control groups using circulating GVII antigen and commercial antigen.

Serological evaluation of the MEVAC ND7 PLUS vaccine 28 days post-vaccination and 7 days post-challenge with commercial antigen:

On day 28 post-vaccination and day 7 post-challenge, blood samples were collected from the remaining chicks in each group to assess Newcastle disease antibody titers. Antibody titers in the MEVAC ND7 PLUS group (8.2 ± 0.7559) and the conventional inactivated vaccine group (8.2 ± 0.8376) did not show a significant difference ($P > 0.8566$). In the positive control group, no chicks survived by day 7 post-challenge (Figure 5(a)).

Serological evaluation of the MEVAC RINNOVAC Eli-7 vaccine 28 days post-vaccination and 7 days post-challenge with commercial antigen:

On day 28 post-vaccination and day 7 post-challenge, blood samples were obtained from the remaining chicks in each group to assess Newcastle antibody titers. Antibody titers in the MEVAC RINNOVAC Eli-7 group (8.5 ± 0.5477) and the Live A vaccine group (5.67 ± 0.5164) did not exhibit a significant difference (P > 0.1828). In the positive control group, no chicks survived by day 7 post-challenge (Figure 5(b)).



	Inactive Conventional	MEVAC ND7 PLUS
Mean	8.200	8.286
Std. Deviation	0.8367	0.7559
Std. Error of Mean	0.3742	0.2857

	MEVAC RINNOVAC Eli-7	Live A
Mean	8.500	5.667
Std. Deviation	0.5477	0.5164
Std. Error of Mean	0.2236	0.2108

Figure 5 (a). Comparison of serological evaluation of the MEVAC ND7 PLUS vaccine 28 days post-vaccination and 7 days post-challenge with circulating virus using commercial antigen. (b) Comparison of serological evaluation of the MEVAC RINNOVAC Eli-7 vaccine 28 days post-vaccination and 7 days post-challenge with circulating virus using commercial antigen.

Evaluation of vaccine uptake on day 5 post-vaccination:

On day 5 after vaccine administration, swab samples were collected from the oral cavity and trachea of chicks receiving the MEVAC RINNOVAC Eli-7 and Live B vaccines. The samples were analyzed using RT-PCR with a Newcastle-specific fusion gene primer. Vaccine detection was negative in all groups.

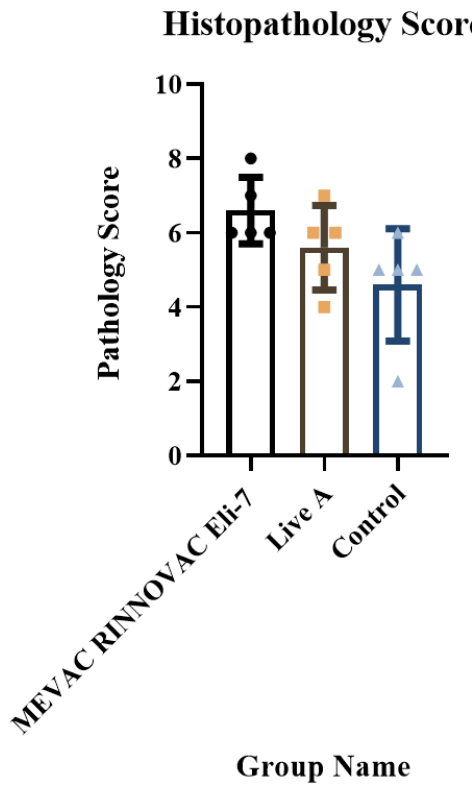
Necropsy findings:

On day 5, all groups exhibited normal trachea, proventriculus, and cecal tonsils upon necropsy. Additionally, all challenged groups were necropsied on day 5 post-challenge, and no lesions were observed in the trachea or proventriculus.

Histopathology:

Histopathological evaluation on day 5 post-vaccination

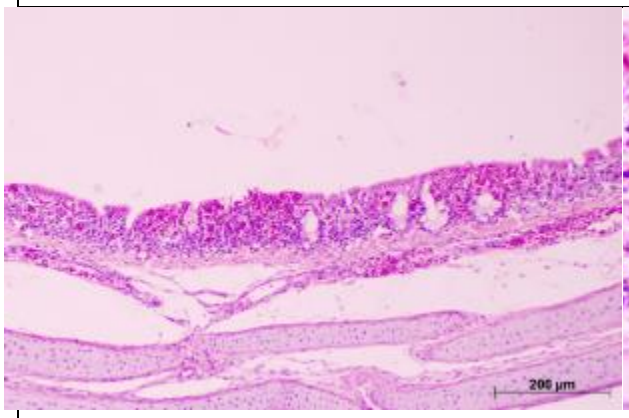
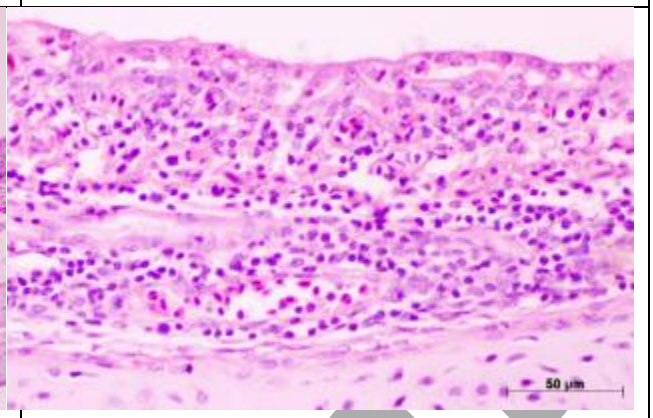
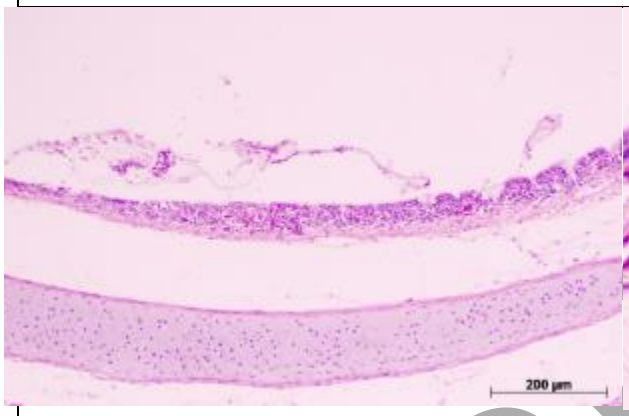
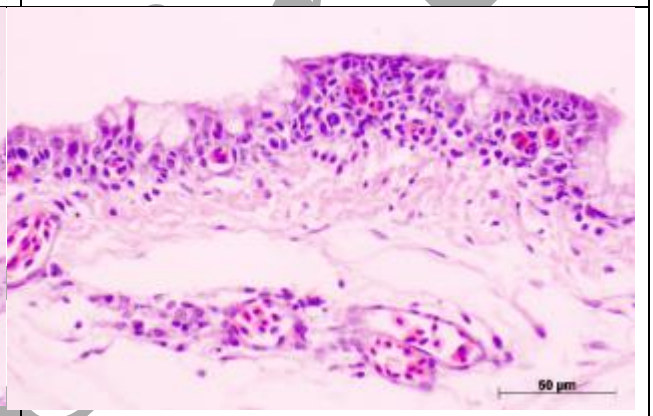
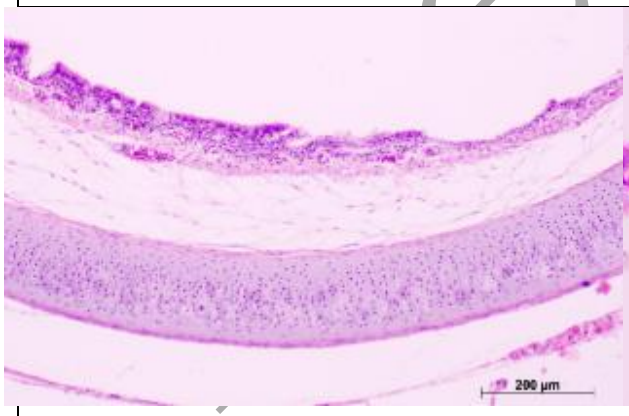
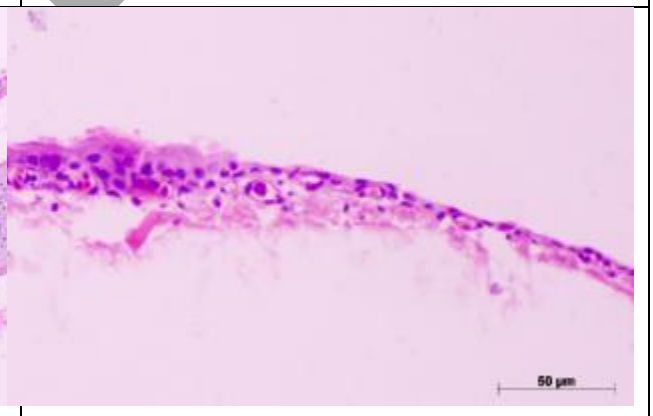
Tracheal tissues were evaluated based on histopathological parameters, including epithelial cell loss, epithelial cell degeneration, lymphoid cell infiltration in the lamina propria, and mucosal and submucosal hemorrhage and hyperemia. After scoring, statistical analysis was performed using the Kruskal-Wallis test. The pathology score in the MEVAC RINNOVAC Eli-7 group (6.6 ± 0.894) did not differ significantly from the Live A vaccine group (5.60 ± 1.140) ($P > 0.5572$). A significant increase in pathology score was observed in the MEVAC RINNOVAC Eli-7 group compared to the negative control group (4.6 ± 1.517) ($P < 0.0446$), whereas the Live A vaccine group did not differ significantly from the control.



	MEVAC RINNOVAC Eli-7	Live A	Control
Mean	6.600	5.600	4.600
Std. Deviation	0.8944	1.140	1.517
Std. Error of Mean	0.4000	0.5099	0.6782

Figure 6. Statistical analysis results of histopathological sections in the two vaccinated groups and the control group.

Figure 7. Histopathological images of tracheal sections in different groups, evaluated at various magnifications.

MEVAC RINNOVAC Eli-7 Group 10X	MEVAC RINNOVAC Eli-7 Group 40X
	
Live A Group 10X	**Live A Group 40X**
	
Control Group 10X	**Control Group 40X**
	

Evaluation of viral shedding at 26 days post-vaccination with MEVAC RINNOVAC Eli-7 and 5 days post-challenge with circulating NDV genotype VII.

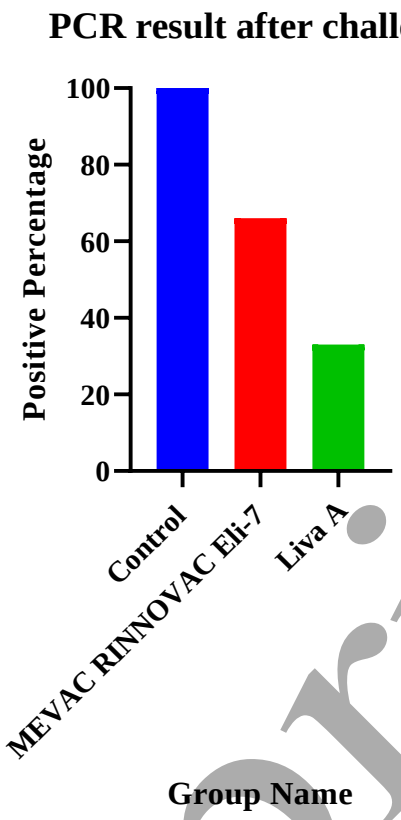
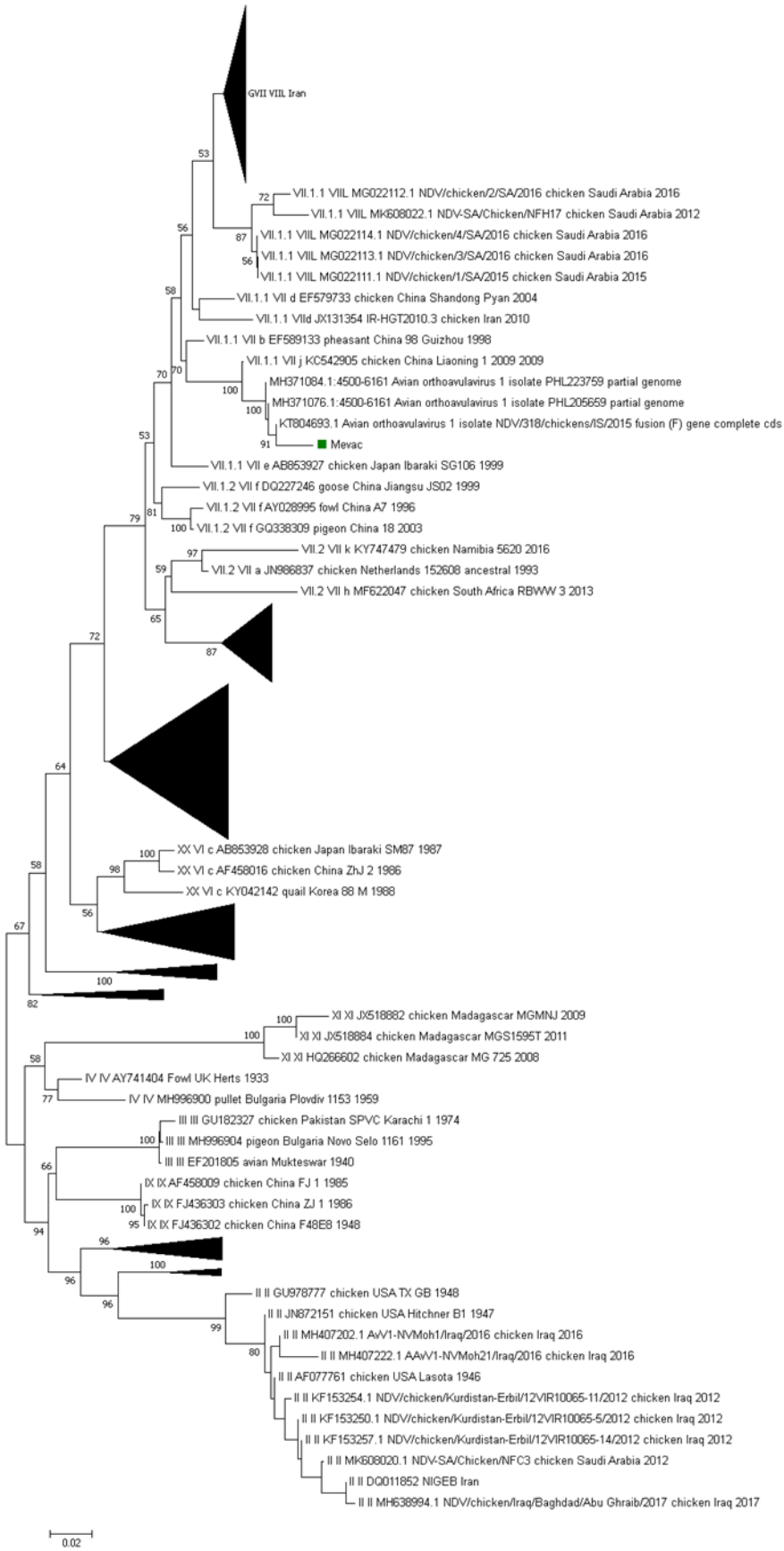


Figure 8. Evaluation of viral shedding at 26 days post-vaccination and 5 days post-challenge with a circulating NDV genotype VII strain.

Phylogenetic evaluation of the vaccine strain in comparison with circulating NDV strains in Iran:

The results of the phylogenetic analysis demonstrated that the live vaccine strain MEVAC RINNOVAC Eli-7 clustered within genotype VII and, according to the previous classification system, belonged to subgenotype VIIj. These findings confirmed the genetic consistency of the vaccine strain with genotype VII NDV strains circulating in Iran (Figure 9).


GVII VIIL Iran
VII.1.1 VIIL MG022112.1 NDV/chicken/2/SA/2016 chicken Saudi Arabia 2016
VII.1.1 VIIL MK608022.1 NDV-SA/Chicken/NFH17 chicken Saudi Arabia 2012
VII.1.1 VIIL MG022114.1 NDV/chicken/4/SA/2016 chicken Saudi Arabia 2016
VII.1.1 VIIL MG022113.1 NDV/chicken/3/SA/2016 chicken Saudi Arabia 2016
VII.1.1 VIIL MG022111.1 NDV/chicken/1/SA/2015 chicken Saudi Arabia 2015
VII.1.1 VII d EF579733 chicken China Shandong Pyan 2004
VII.1.1 VIId JX131354 IR-HGT2010.3 chicken Iran 2010
VII.1.1 VII b EF589133 pheasant China 98 Guizhou 1998
VII.1.1 VII j KC542905 chicken China Liaoning 1 2009 2009
MH371084.1:4500-6161 Avian orthoavulavirus 1 isolate PHL223759 partial genome
MH371076.1:4500-6161 Avian orthoavulavirus 1 isolate PHL205659 partial genome
KT804693.1 Avian orthoavulavirus 1 isolate NDV/318/chickens/IS/2015 fusion (F) gene complete cds
Mevac
VII.1.1 VII e AB853927 chicken Japan Ibaraki SG106 1999
VII.1.2 VII f DQ227246 goose China Jiangsu JS02 1999
VII.1.2 VII f AY028995 fowl China A7 1996
VII.1.2 VII f GQ338309 pigeon China 18 2003
VII.2 VII k KY747479 chicken Namibia 5620 2016
VII.2 VII a JN986837 chicken Netherlands 152608 ancestral 1993
VII.2 VII h MF622047 chicken South Africa RBWW 3 2013
XX VI c AB853928 chicken Japan Ibaraki SM87 1987
XX VI c AF458016 chicken China ZhJ 2 1986
XX VI c KY042142 quail Korea 88 M 1988
XI XI JX518882 chicken Madagascar MGMNJ 2009
XI XI JX518884 chicken Madagascar MGS1595T 2011
XI XI HQ266602 chicken Madagascar MG 725 2008
IV IV AY741404 Fowl UK Herts 1933
IV IV MH996900 pullet Bulgaria Plovdiv 1153 1959
III III GU182327 chicken Pakistan SPVC Karachi 1 1974
III III MH996904 pigeon Bulgaria Novo Selo 1161 1995
III III EF201805 avian Mukteswar 1940
IX IX AF458009 chicken China FJ 1 1985
IX IX FJ436303 chicken China ZJ 1 1986
IX IX FJ436302 chicken China F48E8 1948
II II GU978777 chicken USA TX GB 1948
II II JN872151 chicken USA Hitchner B1 1947
II II MH407202.1 AvV1-NVMoh1/Iraq/2016 chicken Iraq 2016
II II MH407222.1 AAvV1-NVMoh21/Iraq/2016 chicken Iraq 2016
II II AF077761 chicken USA Lasota 1946
II II KF153254.1 NDV/chicken/Kurdistan-Erbil/12VIR10065-11/2012 chicken Iraq 2012
II II KF153250.1 NDV/chicken/Kurdistan-Erbil/12VIR10065-5/2012 chicken Iraq 2012
II II KF153257.1 NDV/chicken/Kurdistan-Erbil/12VIR10065-14/2012 chicken Iraq 2012
II II MK608020.1 NDV-SA/Chicken/NFC3 chicken Saudi Arabia 2012
II II DQ011852 NIGEB Iran
II II MH638994.1 NDV/chicken/Iraq/Baghdad/Abu Ghraib/2017 chicken Iraq 2017
0.02

339

Figure 9. Phylogenetic tree of circulating NDV strains and the MEVAC vaccine strain based on the fusion (F) gene, constructed using the Maximum Likelihood (ML) method with the General Time Reversible (GTR) model and 1000 bootstrap replicates in MEGA 7 software. The MEVAC vaccine strain is indicated by a green square within the tree.

Discussion:

Newcastle disease (ND) remains one of the most highly infectious viral diseases affecting poultry worldwide, particularly in regions where virulent strains circulate endemically [13]. Among Newcastle disease virus (NDV) genotypes, genotype VII (GVII), especially sub-genotype VII.1.1, has emerged as the predominant and most devastating lineage, responsible for severe outbreaks across Asia, Africa, and parts of Europe, and has remained dominant in the Middle East, including Iran, over the past decade [8]. Alarmingly, GVII isolates have been reported to cause mortality rates of up to 100% even in vaccinated flocks, highlighting the urgent need for more effective and targeted control strategies [14, 15].

This study demonstrates that MEVAC RINNOVAC Eli-7 and MEVAC ND7 PLUS vaccines provide strong protective immunity against a virulent NDV GVII challenge. Vaccinated birds developed robust humoral immune responses, with significant increases in HI antibody titres by day 21 post-vaccination, consistent with previous reports indicating that appropriate vaccines can elicit adequate HI responses within this period [16]. Both recombinant live and inactivated vaccines induced strong serological responses, with the reverse genetics–based live vaccine performing equally well or slightly better in some parameters. The maintenance of high antibody titres following challenge further suggests the establishment of sustained immune protection.

A critical parameter for evaluating Newcastle Disease (ND) vaccines is their ability to reduce viral shedding, thereby limiting environmental contamination and the risk of further outbreaks. In this study, the virulent Genotype VII (GVII) challenge resulted in 100% shedding in the Positive Control group, confirming the high susceptibility of unvaccinated birds [17, 18]. Interestingly, while both vaccination programs reduced shedding, the Conventional Live (Live A) vaccine group showed a higher reduction despite the genotype mismatch. This may be attributed to the rapid onset of local mucosal immunity or differences in the viral replication kinetics of the vaccine strains within the respiratory tract [18]. While conventional Genotype II vaccines like LaSota can significantly reduce shedding levels through broad-spectrum cross-reactivity, they may not always

reach the threshold of sterilizing immunity required to eliminate the virus entirely [19]. The shedding observed in the MEVAC Eli-7 group, though higher than the conventional vaccine group in this specific trial, still represents a significant reduction compared to the controls and must be balanced against the vaccine's ability to prevent clinical disease and mortality, which remained high across both vaccinated groups.

A key finding of this study is the clear advantage of genotype-matched vaccination. NDV GVII strains show extensive genetic and antigenic diversity, which can compromise the efficacy of conventional genotype II vaccines such as LaSota [19, 20]. While these traditional vaccines may reduce the severity of clinical disease, they often do not fully prevent viral replication and shedding. In contrast, genotype-matched vaccines provided near-complete protection in this study, with vaccinated birds showing minimal clinical signs and only mild histopathological changes, whereas unvaccinated control birds succumbed to infection. These findings are consistent with previous studies demonstrating that antigenically matched vaccines induce stronger humoral responses and provide improved control of viral dissemination compared with heterologous vaccines [9, 12, 21].

Conclusion

Overall, these results emphasise that phylogenetically informed vaccine design, antigenic matching, and the use of advanced vaccine platforms such as reverse genetics-based live vaccines are critical for optimising NDV control. Implementing genotype-matched vaccination strategies can enhance protective immunity, limit disease severity, and support more effective control of virulent GVII strains in endemic regions.

Conflict of interest

Hereby all authors declare that there is no conflict of Interest.

Ethics

The Institutional Animal Care and Ethics Committee of [Faculty of Veterinary Medicine University of Tehran] examined and approved the study in compliance with the ethical standards

of animal research. Before sample collection, informed consent was obtained from the farms' owners; all the procedures adhered to national and international guidelines on animal welfare.

Data availability

All data analysis is available upon request from the corresponding author.

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Authors Contributions

Acquisition of data: MMA

Analysis and interpretation of data: ZZK, MA, MHFM, MMA

Drafting of the manuscript: MMA, ZZK

Study concept and design: AGL

Study supervision: AGL

All authors reviewed the manuscript.

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