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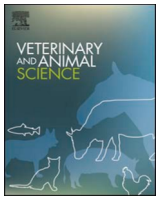

Evaluation of the Newcastle disease virus genotype VII–mismatched vaccines in SPF chickens: A challenge efficacy study

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ABSTRACT

Newcastle disease virus (NDV) strains, while falling under a single serotype, are classified into distinct genotypes. Genotype VII virulent NDVs pose a significant threat to poultry due to their association with high mortality rates and economic losses. This study aimed to evaluate the efficacy of three commercial live vaccines based on genotype II against genotype VII virulent NDV (vNDV) in specific pathogen-free (SPF) chickens. Forty one-day-old chickens were randomly divided into four groups ($n = 10$) and inoculated with one dose of each ND pneumotropic vaccine—B1, Clone.12IR, and La Sota—or received phosphate-buffered saline (PBS) as a control at eight days of age via eye drop. At 28 days of age (20th post-vaccination days), chickens were intramuscularly challenged with genotype VII virulent NDV ($\geq 10^5$ LD_{50}). Serum samples were collected at 28 days of age (challenge day), 7 and 14 post-challenge days to measure NDV antibodies via the hemagglutination inhibition (HI) test. Cloacal and oropharyngeal swabs were taken on the 3rd, 5th, 7th, and 10th post-challenge days to evaluate virus shedding. Vaccinated groups exhibited significantly higher antibody titers and greater protection levels compared to the control group ($P \leq 0.001$). While HI antibody titer was not different at 28 and 35 days of age between vaccinated chickens, the Clone.12IR groups showed higher HI antibody titer compared to B1 at day 42 of age (9.43 vs. 7.42; $P \leq 0.002$). La Sota and Clone.12IR vaccines demonstrated superior protection against mortality compared to the B1 vaccine (90 %, 80% vs. 60 %, respectively) with 6.0 and 2.67 odds ratio of survivability. All three mismatched vaccines effectively curbed the shedding of virulent genotype VII NDV, with 0 % to 11 % positive cloacal samples up to the 3rd post-challenge day. These findings demonstrate that in the experimental setting, the administration of mismatched ND vaccines, particularly La Sota and Clone.12IR, confer protection against genotype VII virulent NDV and control viral shedding, which can help to develop effective vaccination strategies to mitigate the impact of vNDV outbreaks in the poultry farms.

Introduction

Newcastle disease (ND) stands as a highly contagious and economically impactful viral affliction in the global poultry industry (Perozo et al., 2012). Despite extensive vaccination efforts, the persistent occurrence of ND outbreaks and their detrimental effects on poultry production has captured the attention of researchers in recent decades. Factors contributing to the continued prevalence of ND in vaccinated birds include suboptimal vaccination protocols, concurrent immunosuppressive diseases, and viral mutations altering the biological traits and pathogenicity of the Newcastle disease virus (NDV) (Dortmans et al., 2012; Elfatah et al., 2021).

NDV, or Avian orthoavulavirus 1 (AOAV-1), is reclassified within the Orthoavulavirus genus of the Paramyxoviridae family (International committee on taxonomy of viruses (ICTV, 2019)). Phylogenetic analysis of the fusion protein (F) gene sequence categorizes NDV strains into two classes: class I and class II (Miller et al., 2013). Notably, within the class II NDVs, genotype VII strains have been pivotal in the fourth major panzootic of ND, widely circulating across Asia, Europe, the Middle East, and Africa, establishing themselves as one of the predominant and

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crucial global genotypes (Abd-Ellatieff et al., 2021; Miller et al., 2015). Genotype VII NDV strains have been prevalent in Iranian poultry farms since 2011, emerging as the dominant circulating genotype in various avian species within the country (Goudarzi et al., 2019).

Vaccination stands as the cornerstone strategy for ND control and safeguarding avian populations in endemic regions. Several lentogenic NDV strains, characterized by low virulence, serve as live vaccines for ND management. These vaccines exhibit either respiratory tract tropism (pneumotropic), exemplified by strains like B1 and La Sota, or digestive tract tropism (enterotropic), such as VG/GA. Generally, more immunogenic live vaccines tend to be more virulent in chickens, with pneumotropic viruses often demonstrating higher immunogenicity than their enterotropic counterparts (Kim et al., 2016). Despite their classification under a single (AOAV-1) serotype, substantial antigenic and genetic disparities exist among different NDV genotypes. These genotype variations between vaccine strains and circulating NDVs may significantly impact vaccine efficacy in terms of protection and shedding control (Miller et al., 2007).

While various ND vaccines demonstrate protection against morbidity and mortality, evidence suggests that matched vaccines may exhibit a better protection rate (Sultan et al., 2022) and lower viral shedding compared to mismatched ND vaccines (Cheng et al., 2016; Hu et al., 2011; Miller et al., 2007, 2009). Some studies, however, have reported the effectiveness of combined inactivated genotype II (La Sota) and genotype VII vaccines in reducing virus shedding (Mahmoud et al., 2019). Previous investigations have shown that inoculation with the ND. TR.IR vaccine, a live genotype I-based strain, conferred satisfactory protection against local genotype VII virulent NDV and the Herts 33 strain (Abdoshah et al., 2022; Hassanzadeh et al., 2023).

In many countries, vaccination strategies predominantly incorporate live and/or inactivated genotype II-based vaccines for controlling ND. However, the circulation of genotype-VII vNDV, with highly contagious properties, poses a risk of exposing poultry farms to a mismatched virulent virus. Exploring the efficacy of common mismatched vaccines is crucial for gaining insights into their capability to control circulating vNDV as part of vaccination programs and suggesting more accurate ND prevention strategies. To date, comprehensive information regarding the efficacy of genotype II-based ND vaccines in protecting against and reducing viral shedding upon challenge with genotype VII virulent NDV remains elusive. Therefore, the objective of this study was to assess the efficacy of three genotype-II based pneumotropic ND vaccines in protecting against challenges with genotype VII vNDV in chickens.

Materials and methods

The study and the use of animals in the experiments were carried out with the approval of the Ethical Committee of Razi Vaccine and Serum Research Institute (RVSRI.REC.98.024) in Karaj, Iran.

Experimental design and bird management

Forty one-day-old SPF chickens were randomly divided into four groups ($n = 10$). The animal experiments, including virulent NDV challenge and blood sampling, were conducted in a Biosafety Level 3 facility using a BioFlexTM B40 Rigid Body Poultry Isolator (Bell Isolation Systems Ltd, United Kingdom). The Isolator provides controlled housing conditions for poultry, maintaining temperatures ranging from 25 to 30 °C and relative humidity ranging from 40 % to 60 %, adjusting based on the birds' age. Additionally, the isolator provides optimized lighting conditions of 12 h of light per day to ensure the well-being of the birds.

At 8 days of age, the chickens were vaccinated with one of three live genotype II-based ND vaccines: B1, Clone.12IR, La Sota, or received phosphate-buffered saline (PBS) as a control. Vaccines were provided by the Razi Vaccine and Serum Research Institute of Iran and administered one dose ($\geq 10^6$ EID_{50}) per chick via eye drops. For vaccination, each 2500 doses of vaccine vials were reconstituted with 50 mL of PBS, and 20 μL of prepared vaccine was dropped into each chicken's eye. Throughout the 42-day experimental period, chickens had ad libitum access to a standard diet to meet their nutritional requirements including 3000 Kcal ME/Kg, 20 % crude protein, 1 % Ca, 0.5 % available phosphorous (NRC, 2016), and freshwater.

All three vaccines used in this study were manufactured from pneumotropic strains, containing $\geq 10^6$ EID_{50} vaccinal virus but their pathogenicity was different. The B1 and La Sota vaccines provided from international strains showing respiratory tract (pneumotropic) tropism. The Clone.12IR is a commercial vaccine containing a subpopulation of La Sota strain. The intracerebral pathogenicity index (ICPI) of the Clone.12IR is 0.32 that were higher than the B1 (0.18) and lower than the La Sota ICPI (0.40).

Challenge efficacy test

The efficacy test protocol used in this study followed World Organisation for Animal Health terrestrial manual (WOAH, 2019). The velogenic strain CK/IR/ABD/2018, belonging to genotype VII of NDV (accession number: MN615882), was selected for the challenge. This strain was isolated from broiler farms located in the Mazandaran province of Iran. It has been previously characterized as a velogenic NDV with a mean death time (MDT) of 56 h and an ICPI of 1.97. The pathogenicity indices of this strain were found to be similar to those of Hertss 33 challenge virus, which is a standard challenge virus (Hassanzadeh et al. 2023).

The challenge virus was propagated and titrated in SPF-embryonated eggs (Reed & Muench, 1938). All vaccinated (B1, Clone.12IR and La Sota groups) and unvaccinated (control group) chickens were intramuscularly challenged with $\geq 10^5$ LD_{50} of the vNDV (200 μL) on the 20th post-vaccination day and monitored for clinical signs and mortalities during next 14 days (WOAH, 2019).

Laboratory analysis

Hemagglutination inhibition (HI) test

Serum samples from all the birds were collected at 28, 35, and 42 days of age to measure serum antibody titers against NDV using the HI test (WOAH, 2019). The antigen used for serological monitoring originated from the La Sota strain.

Virus shedding

Cloacal and oropharyngeal swabs were collected on the 3rd, 5th, 7th, and 10th post-challenge days. Virus shedding was assessed according to WOAH (2019). Briefly, the swabs were immersed in tubes containing antibiotic (gentamicin, penicillin g, and amphotericin B solutions of 1 mg/ml, 10,000 U/ml, and 20 μg/ml, respectively) for 2 h, and then the supernatant fluids of swabs were harvested following centrifugation at 1000 g for 10 min at 4 to 8 °C. A 200 μL of the supernatant was inoculated into the allantoic cavity of each of the three 10-day SPF embryonated eggs and then were incubated at 37 °C for 7 days. During the incubation period, daily egg candling was performed and those containing dead or dying embryos were chilled to 4 °C overnight, and the allantoic fluids were tested for haemagglutination (HA) activity.

Molecular confirmation of virus-shedding results

Reverse transcription polymerase chain reaction (RT-PCR) was conducted on oropharyngeal and cloacal swabs to confirm negative results by NDV isolation. Viral RNA was extracted using a highly pure viral RNA kit (Roche Life Science, Germany), and cDNA was synthesized with a random hexamer primer. The NDV was used as the positive control. RT-PCR was conducted using a set of primers specific to the fusion (F) protein gene, forward primer 5'-GGTGAGTCTATCCGGARGATACAAG-3' and reverse primer 5'-TCATTGGTTGCRGCAATGCTCT-3' correspond to 202 bp (Creelan et al., 2002). The amplification was carried out with a

final volume of 50 µl containing 32.5 µl of nuclease-free water, 5 µl of 10X PCR Buffer, 2 µl of 50 mM $MgCl_2$, 2 µl of 10 mM dNTP mix, 2 µl of each primer (10 µM), 0.5 µl of 5 units/µl Taq DNA Polymerase (Sinaclon, Iran) and 4 µl of cDNA. The thermocycling conditions were as follows: Initial denaturation at 94 °C for 2 min and 35 cycles of 94 °C for 20 s, 52 °C for 30 s, and 72 °C for 30 s followed by final extension at 72 °C for 5 min. The PCR product was analyzed by electrophoresis in a 1.5 % agarose gel stained with SYBR™ Safe DNA gel stain (Thermo Fisher Scientific, USA).

Statistical analysis

The normality of the HI sera antibody titer was assessed using the Shapiro–Wilk test in SAS 9.4 (SAS, 2004), followed by Analysis of Variance (ANOVA) using the generalized linear model (GLM) procedure. The mortality rate, as a binary distributed measurement, was analyzed using the GENMOD procedure and logit odds ratio link function. The survivability following the challenge test was analyzed using survival analysis and the product limit method of the Kaplan–Meier model utilizing the LIFETEST procedure. Results were presented as mean ± SEM, and Tukey's test was applied for multiple comparisons. Significance levels and tendencies were declared at $P< 0.05$ and $0.05 < P< 0.10$, respectively.

Results

HI antibody titers

Fig. 1 illustrates the mean serum HI titers of vaccinated chickens before the challenge (at 28 days of age) and at 7 and 14 post-challenge days. Vaccinated groups, including B1, Clone.12IR, and La Sota, exhibited significantly higher HI antibody titers compared to the control birds at 28 days of age ($P < 0.05$). There was no significant difference ($P> 0.05$) in antibody titers against ND between chickens vaccinated via B1, Clone.12IR and La Sota vaccines before the challenge (5.14, 6.14, and 5.14, respectively) or at 7 post-challenge days (5.57, 6.71, and 5.86, respectively). However, by the 14th post-challenge day, the Clone.12IR group showed significantly higher ND antibody titers ($P< 0.05$) compared to the B1 group (7.29, 9.43, and 9.00 for B1, Clone 12.IR and La Sota, respectively). Across all vaccinated groups, there was a consistent trend of increased antibody titers at 14 days after the challenge compared to their respective previous titrations ($P< 0.05$).

Vaccine efficacy in challenge test

Clinical symptoms of ND, including depression, drooping wings, and muscular tremors, were observed in all experimental groups within 2 to 3 days post-challenge (Fig. 2). By the 3rd post-challenge day, all chickens in the control group were dead (Table 1). Post-mortem examinations revealed congested visceral organs with hemorrhagic spots on the tip of proventricular glands. In the B1 group, 40 % of the birds died by the 3rd post-challenge day. Conversely, the mortality rate was 20 % in Clone.12IR (two birds on the 5th and 6th post-challenge days) and 10 % in La Sota (one bird on the 3rd post-challenge day). The analysis of survival and mortality rates indicated that the control groups experienced significantly shorter survival times (Fig. 3) and higher mortality rates (100% vs. 40 %, 20 %, and 10 % for the control, B1, Clone.12IR and La Sota, respectively; Table 2). Furthermore, La Sota and Clone.12IR vaccines exhibited 90 % and 80 % protection against mortality due to the virulent VII NDV genotype, with a survivability odds ratio of 6.00

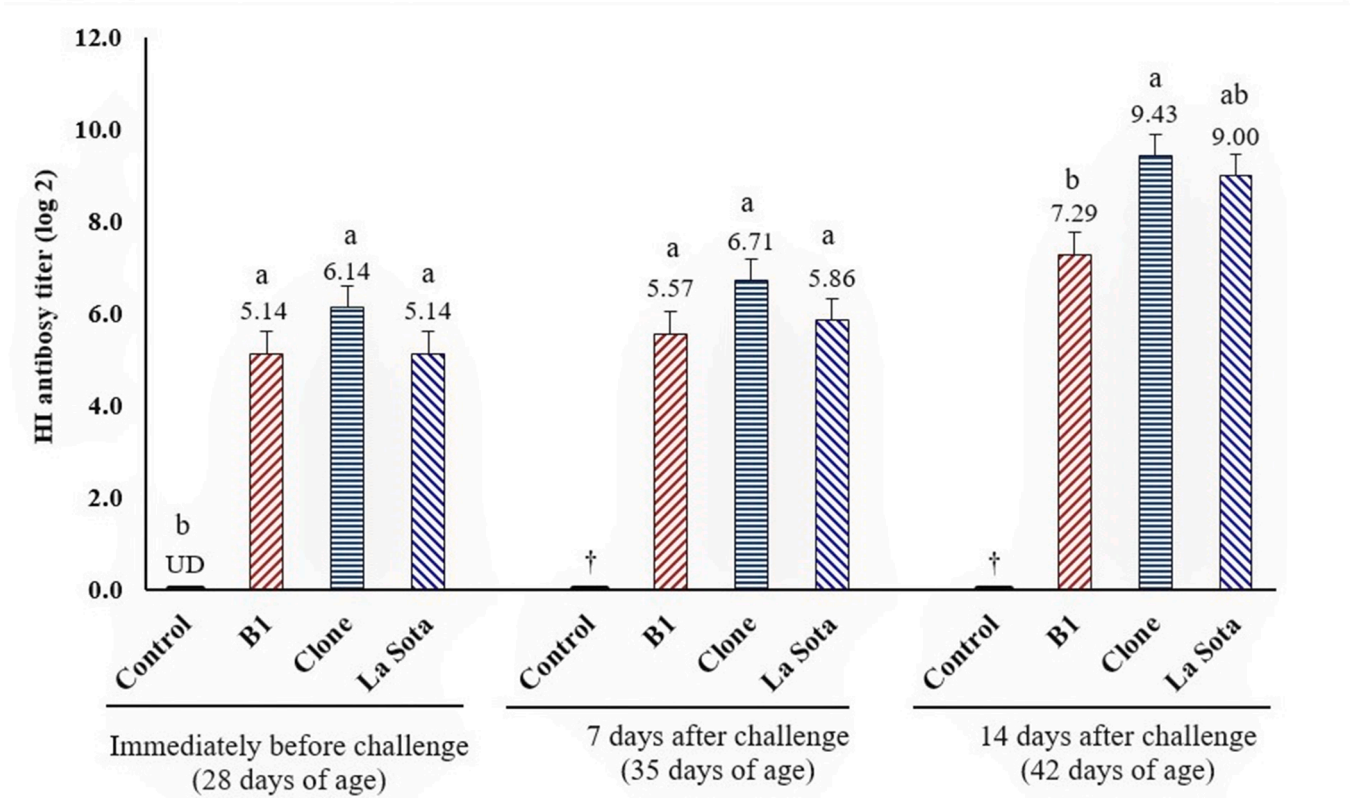

Fig. 1. Mean HI antibody titer (log2) against Newcastle disease (ND) virus in chickens receiving different genotype II-based live ND pneumotropic vaccines challenged with virulent genotype VII NDV.
a, b: Values with different superscripts within each time of blood sampling are significantly different ($P< 0.05$). Note: Specific pathogen-free chickens received B1, Clone.12IR, and La Sota Newcastle disease (ND) vaccines, or phosphate-buffered saline (PBS) as a control, at 8 days of age and were subsequently challenged with virulent ND virus at 28 days of age; UD=undetectable; †: all chickens in the control group had died by three days post challenge (31 days of age).

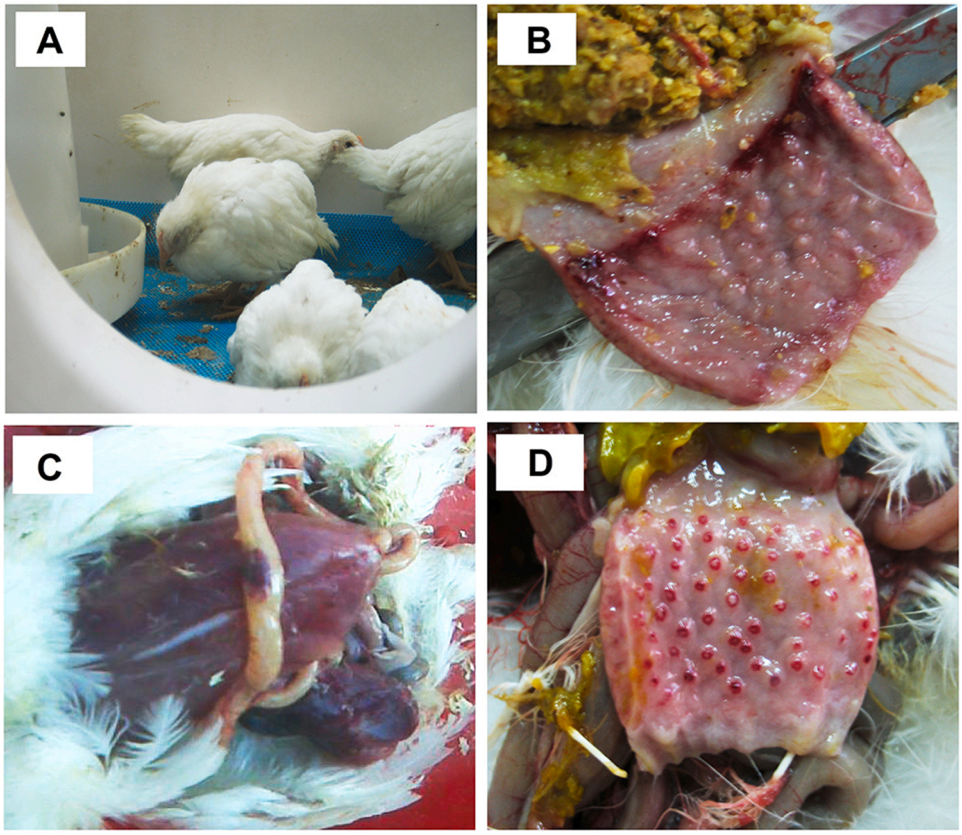

Fig. 2. Clinical symptoms and gross lesions after experimental challenge of specific pathogen-free (SPF) chickens with virulent Newcastle disease virus.
Note: Several signs of Newcastle disease, such as severe depression (A), gastrointestinal lesions between the proventriculus and gizzard (B), jejunal ulcer and congestion of the breast muscle (C), and hemorrhagic lesions on top of the proventricular glands (D), were observed.

Table 1
The onset of clinical signs and days of mortality and protection level of chickens vaccinated with different genotype II-based live pneumotropic Newcastle disease (ND) vaccines challenged with virulent genotype VII NDV ($n = 10$ chickens per group).

Experimental groups *	The onset of clinical signs (days post-challenge)	Birds with clinical signs (%)	Mortality cases on different post-challenge days										Protection rate (%)
			1	2	3	4	5	6	7	8	9	10	
B1	3	40^{b}	–	–	4	–	–	–	–	–	–	–	60^{a}
Clone.12IR	3	20^{b}	–	–	–	–	1	1	–	–	–	–	80^{a}
La Sota	3	10^{b}	–	–	1	–	–	–	–	–	–	–	90^{a}
Control	3	100^{a}	–	–	10	†	†	†	†	†	†	†	0^{b}

a, b: values with different superscripts within each row are significantly different ($P < 0.05$).
* Specific pathogen-free chickens received B1, Clone.12IR, and La Sota vaccines or phosphate-buffered saline (PBS) as control at 8 days of age.
† All the birds died.

and 2.67 compared to the B1 group, respectively (Table 2). In particular, the La Sota group showed a tendency for higher survivability ($P < 0.10$) compared to the B1 vaccine (90% vs. 60 %; Fig. 3). Although the results were not statistically significant ($P > 0.05$), it is noteworthy that the La Sota vaccine exhibited a 2.25 odds ratio compared to Clone.12IR in survivability. This suggests that, in the challenge with genotype VII of NDV, the La Sota strain may confer at least two times more chance for surviving chickens than the Cloned La Sota.

Virus shedding

Table 3 presents the results of challenge virus shedding in chickens vaccinated with different pneumotropic vaccines. Only one cloacal swab from each Clone.12IR and La Sota group (10 % and 11.1 %, respectively) tested positive on the 3rd post-challenge day. Remarkably, all other oropharyngeal or cloacal samples tested in this study showed no virus shedding. Molecular testing for NDV detection confirmed the absence of the virus in all samples that tested negative in the virus isolation test (Fig. 4).

Discussion

Effective vaccines are crucial in controlling ND in poultry alongside biosecurity measures. This study aimed to assess the efficacy of mismatched vaccines against vNDV by examining different genotype II-based live pneumotropic ND vaccines in challenge with genotype VII vNDV. Despite a 23–24 % genetic diversity in F gene sequencing between genotype-II vaccinal viruses and genotype-VII challenge NDV (Hassanzadeh et al., 2023), a notable ≥ 80 % protection was observed in this study.

Key distinctions among ND vaccine strains include tropism and the replication capacity in chickens. The pneumotropic La Sota strain is not only immunogenic but also demonstrates superior replication, resulting in satisfactory neutralizing antibody levels compared to other strains

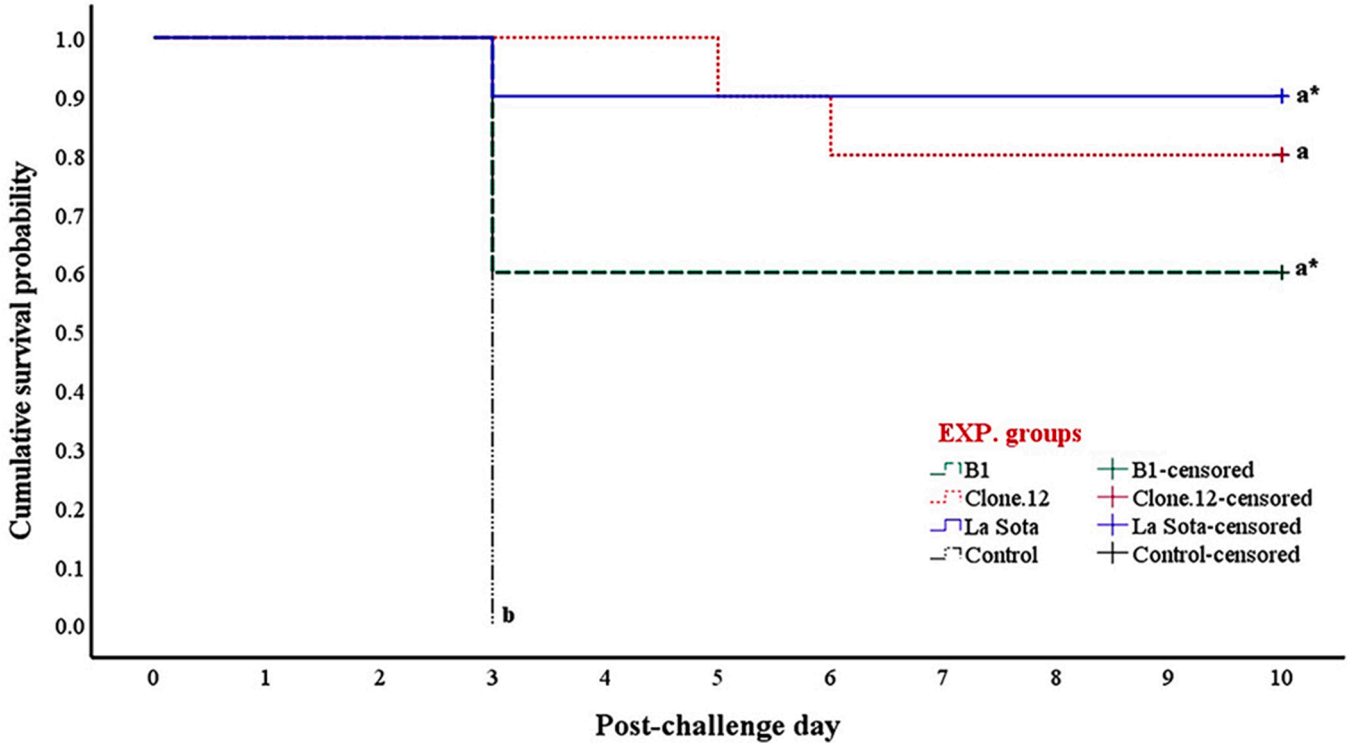

Fig. 3. Survival analysis for specific pathogen-free chickens vaccinated with different pneumotropic genotype-II-based vaccines challenged with virulent genotype VII Newcastle disease virus.
a, b: The experimental groups with different superscripts are significantly different ($P< 0.05$). The experimental groups with an asterisk (*) tended to be significantly different ($0.05 < P< 0.10$). Note: Specific pathogen-free chickens received B1, Clone.12IR, and La Sota vaccines, or phosphate-buffered saline (PBS) as a control at 8 days of age and were subsequently challenged with virulent ND virus at 28 days of age.

Table 2
Odds ratio of survivability in SPF chickens vaccinated with genotype-II based vaccine challenged with genotype-VII virulent virus.

Experimental groups*		Odds Ratio	95 % CI		P value
			Lower	Upper	
La Sota	B1	6.00	0.5	67.65	0.15
La Sota	Clone.12IR	2.25	0.17	29.77	0.54
Clone.12IR	B1	2.67	0.36	19.71	0.34

* Specific pathogen-free chickens received B1, Clone.12IR, and La Sota vaccines or phosphate-buffered saline (PBS) as control at 8 days of age.

(Dimitrov et al., 2017; Omony et al., 2017). In this study, La Sota and Clone12.IR provides 30 % and 20 % more protection rates than the B1 groups resulting in a 6.00 and 2.67 survivability odds ratio. It has been shown that some lentogenic strains, such as La Sota, has heterogeneous subpopulations of virus including both more and less antigenic members that are not always persist. This may explain variations in the immunogenicity of vaccinal strains (Spalatin & Hanson, 1976). Cloned vaccines like Clone12.IR, derived from the La Sota strain, offers a uniform subpopulation with consistent genetic information, inducing high and reproducible immunity with fewer post-vaccinal reactions compared to mixed virus strain vaccines (Ebrahimi et al., 2014). This also may explain the observed longer survivability time and higher odds ratio for protection in La Sota and Colne12.IR compared to the B1 vaccinal strains. Studies have consistently shown genotype II-based vaccines confer high protection against virulent NDV genotype VII strains (Abd-Ellatieff et al., 2021; Roohani et al., 2015; Mahmoud et al., 2019). Dewidar et al. (2022) also demonstrated that the protection level in birds that received two doses of the La Sota live vaccine at 7 and 21 days of age was 93.3 % after the challenge, which was comparable to the group that received the genotype VII recombinant live vaccine.

Table 3
Shedding rate in chickens vaccinated with different genotype II-based live pneumotropic Newcastle disease (ND) vaccines challenged with virulent genotype VII NDV ($n = 10$ at challenge time).

Post challenge day	Samples (swabs)	Experimental groups*			
		B1	Clone.12IR	La Sota	Control
3	Cloaca	0/6	1/10 (10 %)	1/9 (11.1 %)	†
	Oropharynx	0/6	0/10	0/9	†
5	Cloaca	0/6	0/9	0/9	†
	Oropharynx	0/6	0/9	0/9	†
7	Cloaca	0/6	0/8	0/9	†
	Oropharynx	0/6	0/8	0/9	†
10	Cloaca	0/6	0/8	0/9	†
	Oropharynx	0/6	0/8	0/9	†

* Specific pathogen-free chickens received B1, Clone.12IR, and La Sota Newcastle disease (ND) vaccines, or phosphate-buffered saline (PBS) as a control, at 8 days of age and were subsequently challenged with virulent ND virus at 28 days of age.
† : All the birds died at 3rd post challenge day.
Note: the number of birds died in each groups were reported in Table 1.

In the present study, detectable antibody titers from different vaccines at 28 days of age (ranging from 5.14 to 6.14) and 35 (ranging from 5.57 to 6.71) did not differ significantly between vaccinated chickens. However, by the 14th post-challenge day (42 days of age), significant differences emerged between the B1 and Clone.12IR groups, with the

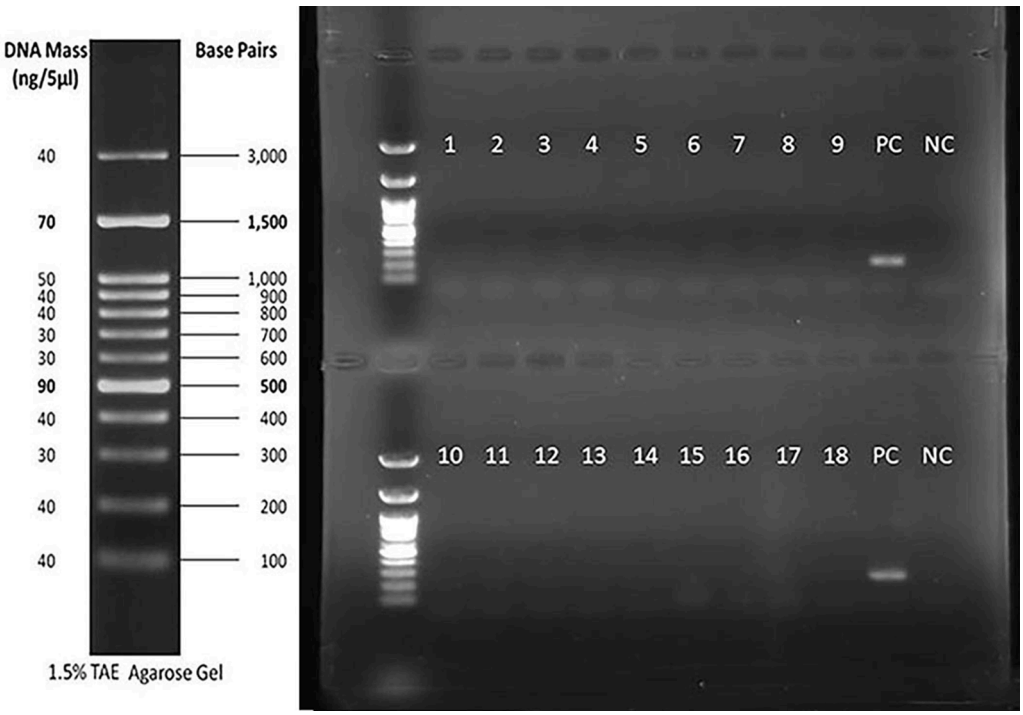

Fig. 4. Gel electrophoresis for the RT-PCR specific to the NDV F protein gene
On the left: 100 bp molecular weight marker.
Lanes 1–9: Cloacal swab samples.
Lanes 10–18: Oropharyngeal swab samples
PC: Newcastle Disease virus as the Positive control (202 bp).
NC: negative control.

Clone.12IR group exhibiting 2.14 $\log_2$ higher HI antibody titers than the B1 group. The trend of the rise of post-challenge antibodies suggests that more than one week is needed to observe a significant increase in HI antibody titers.

The current results showed that pre-challenge HI antibody levels were not different between groups, indicating that the differences observed in protection levels are likely not fully associated with HI antibody titers. It is important to consider that relying solely on humoral immunity responses, such as antibody levels, to predict protection rate may not provide a comprehensive understanding. The correlation between antibody levels and protection or virus shedding is complex and multifaceted. A combination of effects related to levels of antibodies against the F and HN glycoproteins of the virus, local antibodies, and cell-mediated immunity (CMI) are most likely involved in conferring protection against the virus (Reynolds & Maraqa., 2000 a,b).

While some studies suggest that a certain range of HI antibody titers between 4 and 5 log 2 may indicate protective levels, this can vary based on chicken strains, types, and experimental conditions (Liu et al., 2023; Van Boven et al., 2008). Furthermore, multifarious exacerbating conditions (poor feeding management, multipathogen infections, immunosuppressive factors, exposure to a higher viral load or more virulent virus, etc.) that may be encountered in the field can overwhelm immune defenses even at protective antibody levels, leading to increased shedding and mortality (Liu et al., 2023).

Although pre-challenge HI titers resulting from vaccine administration may indirectly reflect the potential for protection, post-challenge titers may not reflect vaccine efficacy, as they are influenced by the vNDV challenge rather than the vaccine antigen itself. Considering HI antibody responses, variations in mortality rates observed in this study could be associated with other aspects of the immune response, such as innate immune responses to virus infection or better CMI, especially with more virulent NDV vaccine strains like La Sota strain (Kapczynski et al., 2013). Therefore, a comprehensive assessment of vaccine efficacy should encompass a broader range of immune responses beyond humoral immunity alone.

In an ND-infected flock, virus transmission to susceptible birds remains the primary mode of disease spread (Miller & Koch, 2020). Hence, reducing virus shedding associated with vaccine efficacy is crucial in disease control in chickens (Palya et al., 2021). It is important to emphasize that molecular methods usually used in shedding evaluation are unable to differentiate between live virus and the presence of virus genome fragments, which may lead to an overestimation of shedding rates. Therefore, the use of virus propagation in embryonated SPF eggs and then confirmation of results by molecular method provides a more reliable evaluation.

In the present study, all three mismatched vaccines effectively prevented vNDV shedding, with only one positive cloacal sample in the Clone.12IR and La Sota groups on the third post-challenge day. The virus shedding in the control or vaccinated chickens was mostly noted during the first post-challenge days. These results were aligned with previous findings demonstrating the highest percentage of shedder birds on the third post-challenge days (Sultan et al., 2020), and also were in agreement with the results showing the ability of mismatched vaccines to prevent virus transmission (Abd-Ellatieff et al., 2021; Miller et al., 2013; Van Boven et al., 2008). However, Miller et al. (2007) and Bello et al. (2020) suggest that challenge vNDV matched vaccines significantly reduce oral shedding compared to mismatched vaccines.

The current study revealed that B1 vaccinated chickens experienced the highest mortality rate, despite no viral shedding compared with the La Sota and Colne.12IR groups. Probably, the death of all birds capable of virus shedding, in the first days after the challenge resulted in the absence of shedder birds in this group. The disassociation between shedding and protection levels observed in this study suggests that the lower shedding rate is valuable if the vaccine also causes an equal or greater protection rate compared to the other vaccines. Therefore, a combined index considering both protection and shedding aspects might

provide a more comprehensive assessment of vaccine efficacy.

The challenge method, including the route of vNDV administration, and the timing of the challenge to vaccination, can significantly influence both protection and shedding rates in chickens. Early challenges may result in increased mortality rates if birds have not yet developed sufficient protective immunity. Under optimal conditions, these live NDV vaccines can achieve a 2 or 3 log reduction in virus shedding. However, this is less likely when early challenges from virulent NDV present in the environment and insufficient biosecurity practices occur (Absalón et al., 2019). Conversely, delaying the challenge might lead to increased mortality if protective immunity levels have waned. In farm conditions, where protective immunity may decrease over time, booster vaccinations are recommended to sustain immunity (Liu et al., 2023; Taylor et al., 2017). In addition, when chickens have not acquired protective immunity and are challenged with vNDV (early infection), significant mortality is expected. Hence, hatchery vaccination is suggested to cover this concern in broiler flocks (Absalón et al., 2019; Hu et al., 2022).

Given the effectiveness of mismatched commercial ND vaccines in this study, the persistence of genotype VII vNDV outbreaks in chicken flocks warrants further investigation. Possible factors contributing to these outbreaks include vaccine delivery failure, vaccination practices, and early infection before vaccine-derived immunity completion, immunosuppressive agents, nutritional deficiencies, or variations in circulating vNDV strains. This study observed international specifications for evaluation of ND vaccines challenge efficacy and hence had a limitation such as a sample size resulting in statistically non-significant between experimental groups; however, further study with a larger sample size is recommended. Additionally, comparing the protection and viral shedding rates of genotype II and VII ND vaccines in challenge efficacy studies can offer further insights into the effectiveness of mismatched vaccines.

Conclusion

In this study, the efficacy of three commercial live vaccines based on genotype II against genotype VII vNDV in SPF chickens was evaluated. Our findings demonstrate that Clone.12IR and La Sota vaccines provided significant protection against high mortality and effectively controlled viral shedding. These findings emphasize the importance of diversifying vaccination strategies for ND prevention and control. By elucidating the effectiveness of genotype II-based vaccines, particularly Clone.12IR and La Sota, our research sets the stage for further investigations aimed at refining vaccination protocols and enhancing ND prevention and control measures. Future studies with larger sample sizes should explore the comparative efficacy of matched and mismatched vaccines, as well as combinations thereof, in challenges with circulating virulent NDV strains. Such research endeavors can provide insights into more effective strategies for controlling ND outbreaks in poultry farms.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly software and a Sider GPT platform to improve readability and language. After using these tools/services, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

CRediT authorship contribution statement

Mohammad Hassanzadeh: Supervision, Conceptualization. **Mehran Abedi:** Investigation. **Mohsen Bashashati:** Visualization, Investigation. **Ali Reza Yousefi:** Writing – original draft, Investigation, Formal analysis. **Mohammad Abdoshah:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Sara Mirzaie:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mohammad Abdoshah reports financial support, administrative support, and equipment, drugs, or supplies were provided by Razi Vaccine and Serum Research Institute. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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