



# Immunogenetic insights into matching bivalent inactivated Payavax G79® for sustainable poultry vaccination against NDV-GVII and H9N2

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## Abstract

Newcastle disease virus (NDV) and H9N2 avian influenza virus (AIV) have a severe impact on the poultry industry. This Iranian study evaluated Payavax G79®, an inactivated bivalent vaccine, in broiler chickens across five provinces. Vaccinated chicks showed robust antibody responses to both viruses via HI tests at 25 and 32 days post-vaccination. The vaccine provided complete protection against challenge with velogenic NDV genotype VII. However, H9N2 infection occurred on three farms, with 2% mortality. Molecular analysis of NDV isolates revealed amino acid substitutions in the hemagglutinin-neuraminidase protein that potentially enhance antigenicity. This study shows that Payavax G79® is highly immunogenic and provides significant protection against both NDV and H9N2 AIV under field conditions in Iran.

**Keywords** Vaccine · Newcastle disease virus · H9N2 avian influenza · Immunogenicity · Effectiveness

Avian influenza virus (AIV) and Newcastle disease virus (NDV) are the two primary viruses that cause avian influenza and Newcastle disease (ND) in chickens. These viruses cause high mortality when they coinfect poultry, decreasing productivity and egg production in affected flocks, resulting in considerable financial losses [1]. NDV can infect a bird's nervous system, visceral organs, and respiratory tract, whereas LPAIV affects mainly the respiratory tract. Thus, even when they survive, birds infected with LPAIV or NDV produce less meat and fewer eggs [2].

Avian paramyxovirus serotype I (APMV-I), also known as Newcastle disease virus (NDV), is a member of the genus *Orthoavulavirus* of the family *Paramyxoviridae*. This virus causes ND, one of the most common diseases of poultry, and results in significant financial losses associated with disease control [3]. The NDV genome is a non-segmented, single-stranded, negative-sense RNA molecule, and NDV isolates exhibit a high degree of genetic diversity. The genome encodes six structural proteins: hemagglutinin-neuraminidase (HN), fusion (F), matrix (M), nucleoprotein (NP), phosphoprotein (P), and large polymerase (L). In addition, two non-structural proteins (V and W) are produced via RNA editing of the *P* gene transcript [4]. According to the genotype classification system of Diel et al. NDV isolates can be categorized into classes I and II, with class II being of greater importance for poultry, as virulent NDVs can cause 100% mortality [5], velogenic Newcastle disease viruses (NDVs) are linked to global poultry outbreaks.

Avian influenza viruses (AIVs) are classified as low-pathogenic avian influenza (LPAI) or highly pathogenic avian influenza (HPAI) strains. LPAI strains cause mild illness but may become severe in coinfections with other pathogens, while HPAI (mainly H5/H7) leads to high mortality, even when it is the only infecting pathogen [6]. Type A avian influenza viruses, belonging to the family *Orthomyxoviridae*, are the main cause of avian influenza. Their

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genomes are made up of eight RNA segments. Genetic changes in AIVs, known as drift and shift, have resulted in 18 hemagglutinin (HA) and 11 neuraminidase (NA) surface glycoprotein subtypes [7]. Like NDV, AIV poses a major threat to poultry health. This threat is particularly evident with H5N1, a highly pathogenic subtype known to cause high mortality, but is also significant with H9N2, H7N3, and H3N2, which often have low pathogenicity but can nevertheless cause economic losses due to decreased egg production or, critically, increased mortality in case of coinfection with other pathogens [8].

In Asia and the Middle East, including Iran [9], Pakistan [10], Kazakhstan [11], Qatar [12], Korea [13], Japan [14], Vietnam [15], and Malaysia [16], ND outbreaks are caused primarily by class II NDVs of genotype VII. Consequently, the substantial financial losses incurred by the poultry sector due to AIV and NDV highlight the need for vaccine development and improvement. Newcastle disease (ND) and avian influenza virus (AIV) outbreaks on commercial poultry farms have been greatly reduced by large-scale vaccination programs and biosecurity measures [17]. A recent decrease in ND outbreaks in China suggests that the current measures are having some effect. However, continuous improvement of vaccine formulas is necessary due to changes in the virus and varying severity. The use of bivalent oil emulsion vaccines is the most efficient way to protect chickens against both avian influenza and Newcastle disease, providing long-lasting protection while saving on labor and reducing the stress caused by administering two separate single-disease vaccines [18].

In this study, we evaluated the immunogenicity and effectiveness of Payavax G79®, an inactivated bivalent vaccine targeting Newcastle disease virus G-VII and H9N2 avian influenza virus (AIV), on 50 farms in different high-risk regions across five Iranian provinces. Ten-day-old broiler chickens received a single 0.2-mL subcutaneous dose of the vaccine, and antibody titres against NDV and H9N2 were measured using a hemagglutination inhibition (HI) test on serum samples collected at 25 and 32 days post-vaccination (dpv), following OIE protocols using 1% chicken red blood cells. Antibody titres are expressed as log<sub>2</sub> values. Vaccine effectiveness against NDV-GVII and H9N2 infection was assessed by testing swab samples collected at 7, 14, 21, 28, and 35 dpv for the presence of the virus. Viral load positivity and mortality within the flocks was also recorded.

The nucleotide sequences of the HN (MZ605768) and F (MN481196) genes from local NDV-VII.1.1 isolates were compared to those of Payavax G79® and five other vaccine strains, including VG/GA (EU289028.1), PHY-LMV42 (DQ09794.1), B1 (AF309418.1), Lasota (AF077761.1), and V4 (AY225110.1), using DNASTar and MEGA X software. Amino acid sequence alignments of the HN and F

proteins were generated using CLC Genomics Viewer 8.0 (Figs. 1 and 2). Three-dimensional (3D) structures of the surface glycoproteins of the local NDV isolates, Payavax G79®, and the Lasota strain (AF077761.1) were modelled using Swiss-Model [19, 20], based on the protein ectodomain structures of homologous HN (PDB: 7BWU, 3T1E) and F (PDB: 3MAW, 1G5G) proteins (Fig. 3). Sequence alignments and 3D structure comparisons were performed using PyMOL 3.1.1 software. Statistical analysis was performed using GraphPad Prism 10. Depending on the data structure, one-way ANOVA and descriptive statistical tests were applied as appropriate. Statistical significance was defined as  $p < 0.05$ .

Serum samples collected from vaccinated broilers at 25 and 32 dpv showed that Payavax G79® elicited a rapid and statistically significant increase in HI titer ( $p < 0.05$  at both time points when compared to pre-vaccination levels). The mean HI titer at 25 days was 6.6 log<sub>2</sub> for NDV and 4 log<sub>2</sub> for H9N2, peaking at 32 days to 7.2 log<sub>2</sub> for NDV ( $p < 0.01$ ) and 4.6 log<sub>2</sub> for H9N2 ( $p < 0.05$ ) (Supplementary data 1).

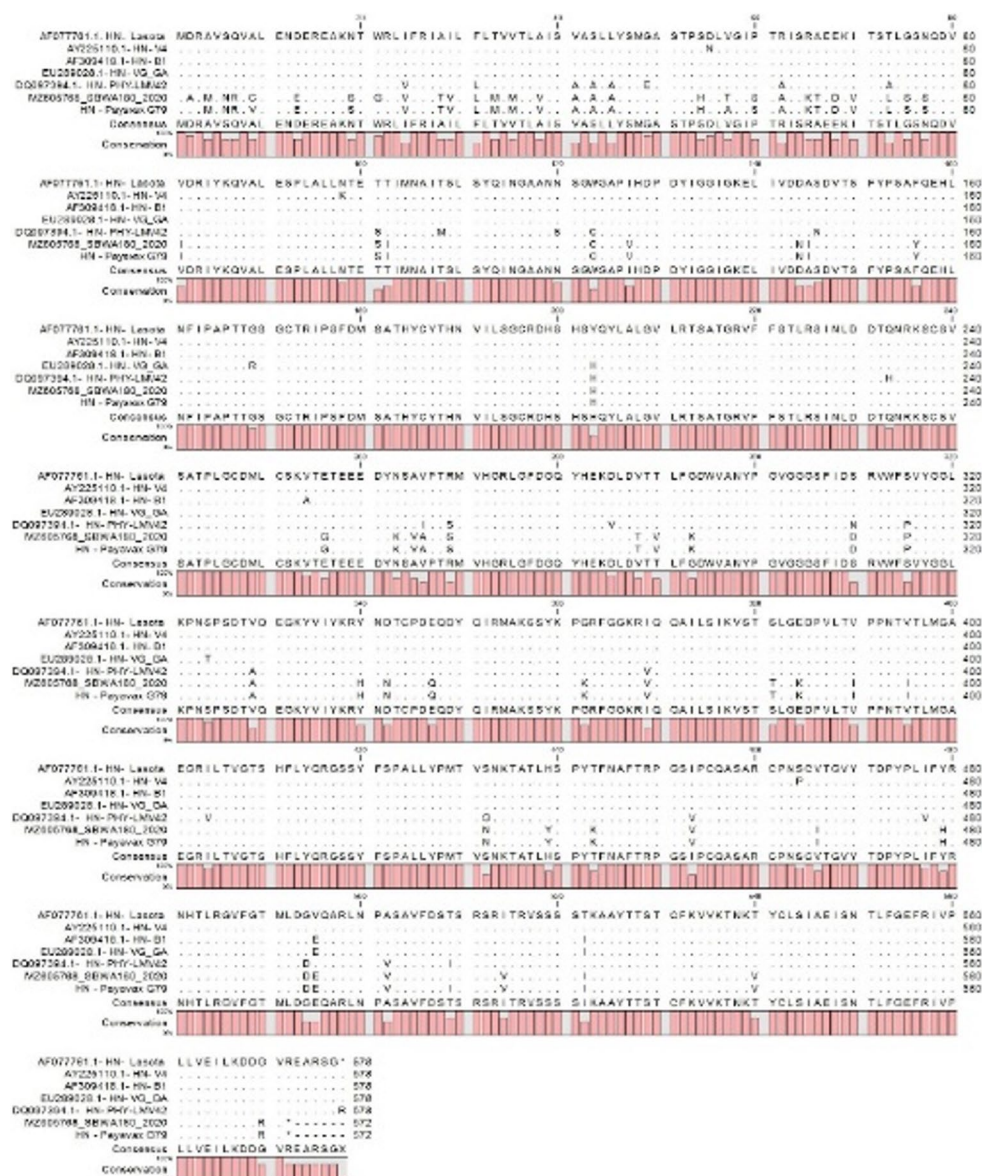
As shown in Supplementary data 2, a significant increase in mortality was observed at 7 dpv, which subsided by 14 dpv ( $p < 0.05$ ), then gradually rose until 35 dpv. Based on the correlates of protection – HI titers  $\geq 5$  log<sub>2</sub> for NDV-GVII and  $\geq 4$  log<sub>2</sub> for H9N2 – the vaccinated birds exhibited adequate immunogenic responses.

A minor outbreak of H9N2 infection on one of the 50 farms studied, where 1.6% of the 20,000 broilers showed respiratory signs and mortality around 30 dpv, but no NDV-GVII infections were detected by PCR. The difference was significant when compared to controls ( $p < 0.01$ ), indicating that Payavax G79® conferred significant protection (Supplementary data 2). An alignment of the predicted HN sequences of a VII.1.1 isolate (MZ605768), Payavax G79®, and five common vaccine strains (VG/GA, PHY-LMV42, B1, Lasota, and V4) revealed that Payavax G79® contains a new N-linked glycosylation site (N-I-S, aa 144–146) that is present in VII.1.1 [20] but absent in the other vaccine strains (Fig. 1). Further substitutions (I369V, V329A, and S315P; Fig. 1) present in both VII.1.1 and Payavax G79® have been suggested to affect the thermostability of VII.1.1 [20].

Antigenic [21] and sialic-acid-binding sites [22] on HN showed differences between VII.1.1/Payavax G79® and the vaccine strains. Three sialic acid binding site substitutions (F156Y, Y203H, and T522I; Fig. 1) differentiate field isolates from vaccines. The antigenic site substitutions E347Q [23, 24], G494D, and I514V [25] (Fig. 1) in VII.1.1 might alter HN antigenicity. Payavax G79® contains all of these substitutions, whereas the common vaccine strains do not (Fig. 1).

A comparison of the F protein sequences of VII.1.1 (MN481196), Payavax G79®, and the five vaccine strains

**Fig. 1** Alignment of the predicted amino acid sequences of the HN proteins of the NDV isolate (MZ605768), Payavax G79®, and five commonly used vaccine strains (VG/GA, PHY-LMV42, B1, Lasota, and V4), performed using CLC sequence viewer 8.00



showed that the F0 cleavage site is 112RRQKRF117 in both VII.1.1 and Payavax G79®, differing from the 112GRQGRL117 cleavage site in the vaccine strains (Fig. 2). In the HR2 region, the substitutions N479D and R486S in both VII.1.1 and Payavax G79® are linked to enhanced NDV fusion activity [26] (Fig. 2).

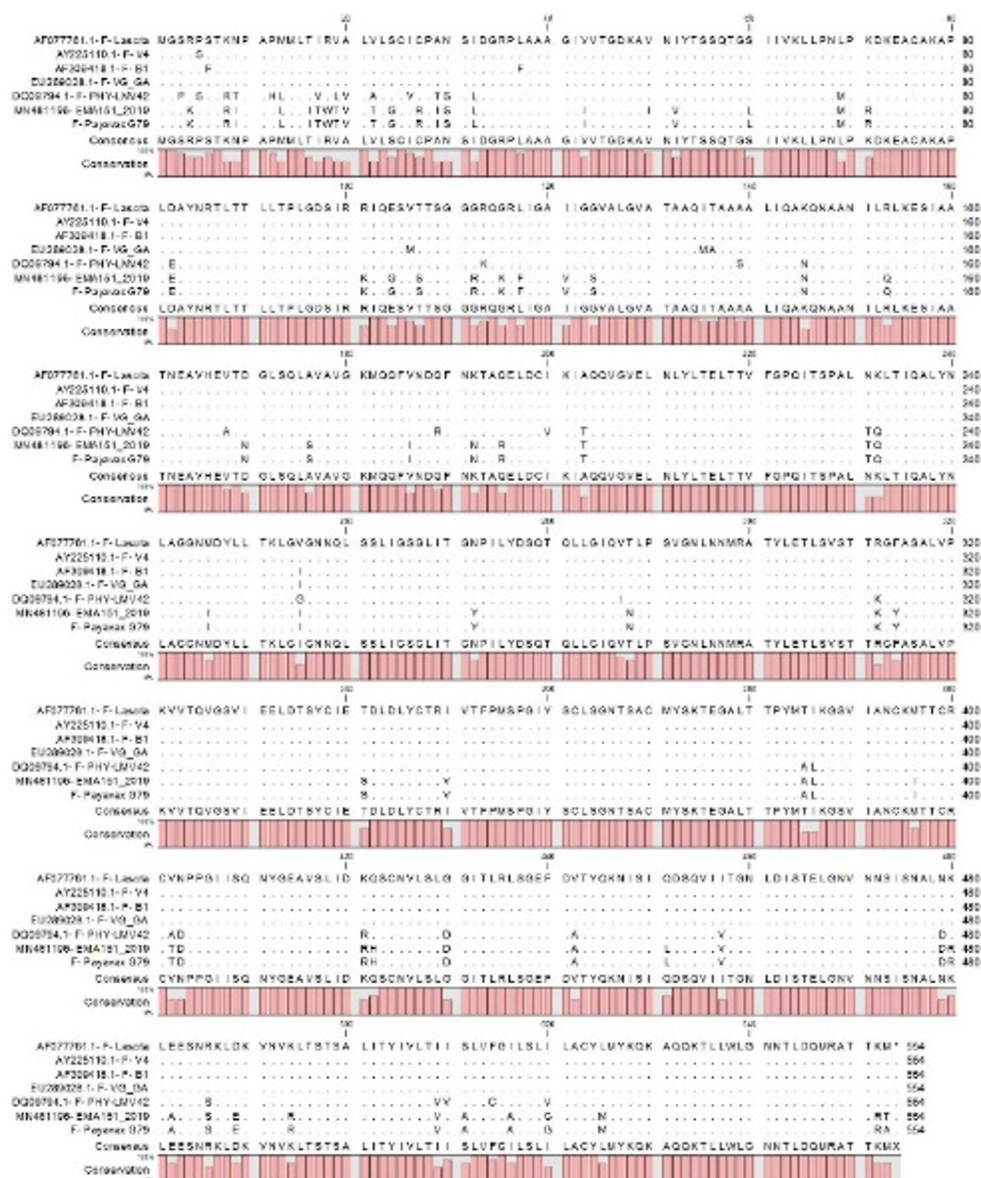
3D structural predictions of the surface glycoproteins of VII.1.1 (HN: MZ605768; F: MN481196), Payavax G79®, and strain Lasota indicated close alignment of the Payavax G79® HN and P proteins with recent Iranian wild NDV isolates, particularly compared to Lasota (Fig. 3). Previous research has demonstrated that changes in the 3D structures of NDV proteins affect the virus's virulence [27]. Thus, the strong similarity of the HN and F proteins of the wild isolates and Payavax G79® could lead to promising outcomes in practical applications [27].

The rapid evolution of NDV has led to the emergence of numerous genotypes and subgenotypes globally [28]. The genomes of RNA and DNA viruses can undergo recombination, generating new variants. In Iran, genotype VII.1.1, which is found in various avian species, appears to be the prevalent NDV genotype [29]. Isolates of this genotype elicit immune responses that differ from those induced by genotype-II-based vaccines, and this might explain why ND outbreaks continue to occur in vaccinated chickens [30]. Updated vaccines matching the circulating viruses are being developed, and Payavax G79®, an inactivated bivalent vaccine targeting NDV G-VII and H9N2 avian influenza virus (AIV), was evaluated in this study.

Payavax G79® demonstrated significantly higher protection rates and lower mortality against virulent NDV-GVII compared to historical controls using heterologous vaccines



**Fig. 2** Alignment of the predicted amino acid sequences of the F proteins of the NDV isolate (MN481196), Payavax G79®, and five commonly used vaccine strains (VG/GA, PHY-LMV42, B1, Lasota, and V4), performed using CLC sequence viewer 8.00



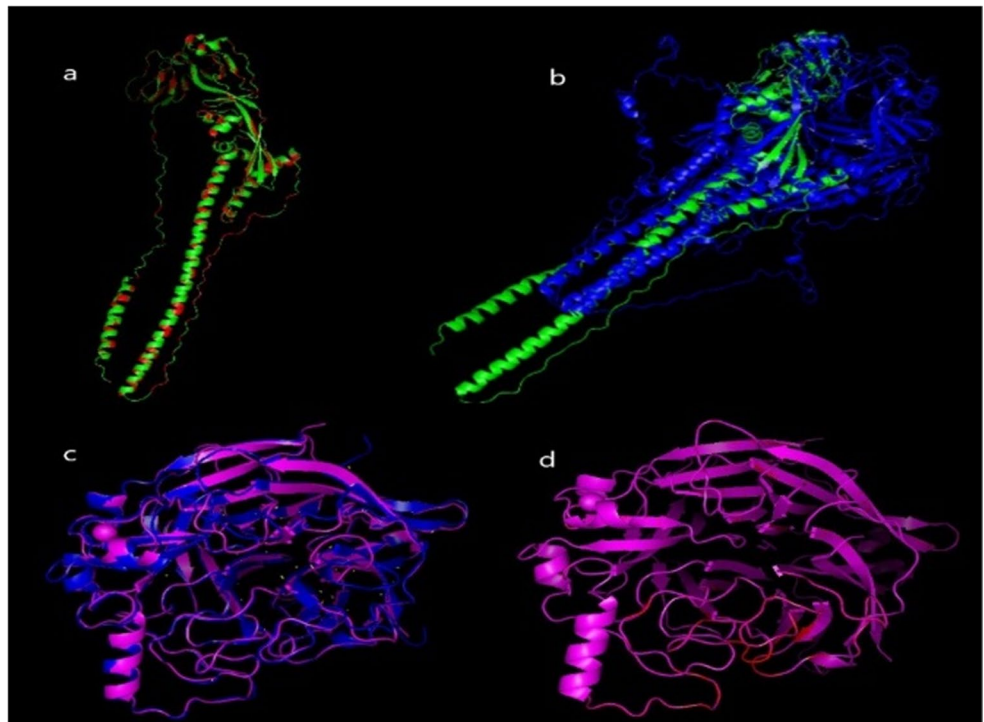
( $p < 0.01$ ). The vaccine's ability to elicit a rapid, robust, and sustained immune response was found to be statistically significant ( $p < 0.05$ ), underscoring its potential for controlling NDV and H9N2 in endemic regions.

HI antibody titers are key indicators of vaccine immunogenicity. Payavax G79® induced a rapid HI antibody response that was stronger than those induced by conventional vaccines, likely due to its antigenic similarity to circulating NDV and H9N2 strains in Iran. In our previously published study [31], we compared the HI antibody responses induced by various commercial NDV vaccines from different sub-strains, showing superior immunogenicity of genotype-VII-based vaccines. Notably, the flocks in the current study were located in regions that were hotspots for NDV-GVII and AIV-H9N2 strains, and complete viral

clearance was observed post-vaccination, confirming the candidate vaccine's field efficacy.

Seven overlapping antigenic sites on the HN glycoprotein affect virion attachment and neutralize infection [17]. Subgenotype VII.1.1 and Payavax G79® both contain isoleucine 514→Valine (sites 12 and 2) and glutamic acid 347→ glutamine (sites 1 and 14) substitutions. Monoclonal antibodies targeting sites 12, 2, and 23 can inhibit NDV infection [32]. The I514V mutation is found in virulent isolates [25]. Changes at residue 347 can alter the antigenicity of the virus, and the E347K mutation has been linked to vaccination failure [33]. A Trp123→Cys substitution in HN was also detected in VII.1.1 and Payavax G79®. Cysteine residues are critical for disulfide linkages in the HN homodimer [34].

**Fig. 3** 3D structural models of the HN and F proteins of the VII.1.1 NDV isolate (NCBI accession numbers MZ605768 for HN and MN481196 for F) constructed using the SWISS-MODEL online software for 3D predictions, based on protein alignments performed using PyMOL 3.1.1 software. **(a)** Comparison of the F proteins of the VII.1.1 NDV isolate (shown in green) and Payavax G79® (shown in red). **(b)** Comparison of the F proteins of the VII.1.1 NDV isolate and the Lasota vaccine strain (NCBI accession number AF077761.1) (shown in blue). **(c)** Comparison of the HN proteins of the VII.1.1 NDV isolate (shown in magenta) and Payavax G79® (shown in red). **(d)** Comparison of the HN proteins of the VII.1.1 isolate and the Lasota vaccine strain (shown in blue).



In the F protein, N479D and R486S substitutions in the HR2 region, present in Payavax G79®, but not in common vaccine strains, can enhance fusion activity [27]. Our 3D modeling of the HN and F proteins of Payavax G79® suggest strong structural similarity to currently circulating field isolates.

Key amino acids in VII.1.1 and Payavax G79® compared to standard vaccines include the NIS glycosylation site, I369V, and S315P. The HN protein of NDV is crucial for cell attachment and hemagglutinin-neuraminidase activity [24]. Various amino acid substitutions in HN, including glycosylation sites, antigenic sites, sialic acid binding sites, and cysteine residues, have been shown to influence NDV pathogenesis and thermostability [35]. N-linked glycosylation is vital for HN function, and the gain or loss of a glycosylation site can affect the biological activity and virulence of NDV [36]. The new N-linked glycosylation site (NIS) at positions 144–146 in subgenotype VII.1.1, which is absent in the common vaccine strains, may enhance virulence and HA activity. The HN of Payavax G79® contains this site.

While most NDV genotypes are heat-sensitive, some are thermostable. The mutations S315P, I369V, and V369A were observed in the VII.1.1 isolate. Two of these, S315 and I369V, have been shown to enhance thermostability and HA/NA activity [20]. Proline residues such as that introduced by the S315P mutation, have the potential to increase protein thermostability [37] and possibly to enhance vaccine efficacy.

In summary, the key amino acid changes observed in both the VII.1.1 isolate and Payavax G79®, when compared to standard vaccine strains, include the N-glycosylation site (NIS) at position 144, the I369V and S315P mutations, which enhance viral thermostability, the addition of a cysteine residues at position 123, specific amino acid alterations in the HN antigenic sites, particularly the E347Q and I514V mutations, and the aa substitutions of N479D and R486S in the HR2 region of the F protein, which may improve antigenic coverage and HI titers. Homologous vaccines targeting subgenotype VII have shown better protection against virulent NDV than heterologous vaccines such as LaSota, reducing mortality, morbidity, and virus shedding [38]. Inactivated homologous vaccines also offer superior clinical protection. Incorporating these crucial substitutions is therefore essential for increasing vaccine efficacy [39].

In commercial poultry, Payavax G79® provided complete protection against ND, with no mortality. A minor influenza outbreak with low mortality was observed, but this was considered acceptable in an industrial setting. Consistent with prior research, Payavax G79® provided early and strong immunity against influenza virus subtype H9N2, reducing mortality. It also conferred superior protection against virulent NDV (genotype VII.1.1), with higher protection rates and lower mortality. The enhanced efficacy likely stems from optimized adjuvants or antigen combinations in Payavax G79®, leading to a more potent adaptive immune response.

Payavax G79® is an effective bivalent vaccine that protects broiler chickens from both influenza A virus subtype H9N2 and NDV, potentially reducing financial losses and increasing productivity. All Iranian NDV isolates belong to genotype VII, and homologous vaccines are therefore likely to offer better protection. Further studies with longer follow-up are needed to corroborate these results.

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**Author contribution** P. J: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. S. B: in silico study, study design, formal analysis, writing – original draft, writing – review & editing. N. D. H: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. M. Gh. G: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. R. Sh. M: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. T. M: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. Z. Kh: study design, formal analysis, methodology, investigation, data curation, writing – original draft, writing – review & editing. M. Sh: conceptualization, study design, formal analysis, methodology, investigation, data curation, project management, writing – original draft, writing – review & editing. All of the authors critically reviewed and approved the final version of the manuscript.

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**Data availability** All data generated or analysed in this study are included in the published article.

## Declarations

**Ethical approval** The Iranian Administration Committee of Laboratory Animals (IACLA) accredited the animal-rearing facilities housing the chickens with approval code AVL.ETH.1403.02.

**Conflict of interest** The authors declare that there are no conflicts of interest.

## References

- Costa-Hurtado M et al (2014) Virus interference between H7N2 low pathogenic avian influenza virus and lentogenic Newcastle disease virus in experimental co-infections in chickens and turkeys. *Vet Res* 45(1):1
- Shabbir MZ et al (2013) Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective. *Virol J* 10:1–10
- Mahmood MS, Sabir R (2021) Preparation and evaluation of avian influenza (H9) and Newcastle disease (thermostable i-2 strain) bivalent vaccine for commercial poultry. *Agrobiological Records* 3:17–23
- Cattoli G et al (2011) Newcastle disease: a review of field recognition and current methods of laboratory detection. *J Vet Diagn Invest* 23(4):637–656
- Diel DG et al (2012) Complete genome and clinicopathological characterization of a virulent Newcastle disease virus isolate from South America. *J Clin Microbiol* 50(2):378–387
- Cook RA, Karesh WB (2011) Emerging diseases at the interface of people, domestic animals, and wildlife. *Fowler's Zoo Wild Animal Med*:136
- Sattar A et al (2016) Immunosuppressive effects of arsenic in broiler chicks exposed to Newcastle disease virus. *J Immunotoxicol* 13(6):861–869
- Nagarajan S et al (2009) Isolation and pathotyping of H9N2 avian influenza viruses in Indian poultry. *Vet Microbiol* 133(1–2):154–163
- Boroomand Z, Jafari RA, Mayahi M (2016) Molecular characterization and phylogenetic study of the fusion genes of Newcastle disease virus from the recent outbreaks in Ahvaz, Iran. *Virusdis-ease* 27:102–105
- Munir M, Chowdhury M, Ahmed Z (2016) Emergence of new sub-genotypes of Newcastle disease virus in Pakistan. *J Avian Res* 2(1):1–7
- Bogoyavlenskiy A et al (2009) Newcastle disease outbreaks in Kazakhstan and Kyrgyzstan during 1998, 2000, 2001, 2003, 2004, and 2005 were caused by viruses of the genotypes VIIb and VIId. *Virus Genes* 39(1):94–101
- Haroun M et al (2015) Molecular pathotyping and phylogenesis of the first Newcastle disease virus strain isolated from backyard chickens in Qatar. *Trop Anim Health Prod* 47(1):13–19
- Kim B-Y et al (2012) Exchange of Newcastle disease viruses in Korea: the relatedness of isolates between wild birds, live bird markets, poultry farms and neighboring countries. *Infect Genet Evol* 12(2):478–482
- Mase M et al (2011) Analysis of the fusion protein gene of Newcastle disease viruses isolated in Japan. *J Vet Med Sci* 73(1):47–54
- Choi K-S et al (2014) Molecular epidemiology of Newcastle disease viruses in Vietnam. *Trop Anim Health Prod* 46:271–277
- Jaganathan S et al (2015) Observation of risk factors, clinical manifestations and genetic characterization of recent Newcastle Disease Virus outbreak in West Malaysia. *BMC Vet Res* 11:1–9
- Ebrahimi MM et al (2012) Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008–2011. *Virus Genes* 45:63–68
- Park M-S et al (2006) Engineered viral vaccine constructs with dual specificity: avian influenza and Newcastle disease. *Proc Natl Acad Sci* 103(21):8203–8208
- Arnold K et al (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 22(2):195–201
- Ruan B et al (2020) Residues 315 and 369 in HN protein contribute to the thermostability of Newcastle disease virus. *Front Microbiol* 11:560482
- Iorio RM, Bratt M (1984) Monoclonal antibodies as functional probes of the HN glycoprotein of Newcastle disease virus: antigenic separation of the hemagglutinating and neuraminidase sites. *J Immunol* 133(4):2215–2219
- Jorgensen ED, Collins PL, Lomedico PT (1987) Cloning and nucleotide sequence of Newcastle disease virus hemagglutinin-neuraminidase mRNA: identification of a putative sialic acid binding site. *Virology* 156(1):12–24

23. Zhu J et al (2016) Characterization of virulent Newcastle disease viruses from vaccinated chicken flocks in Eastern China. *BMC Vet Res* 12(1):113
24. Hu S et al (2010) Identification of a variable epitope on the Newcastle disease virus hemagglutinin–neuraminidase protein. *Vet Microbiol* 140(1–2):92–97
25. Seal BS (2004) Nucleotide and predicted amino acid sequence analysis of the fusion protein and hemagglutinin-neuraminidase protein genes among Newcastle disease virus isolates. Phylogenetic relationships among the Paramyxovirinae based on attachment glycoprotein sequences. *Funct Integr Genom* 4(4):246–257
26. Gravel KA, Morrison TG (2003) Interacting domains of the HN and F proteins of Newcastle disease virus. *J Virol* 77(20):11040–11049
27. Chen L et al (2001) The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrane fusion. *Structure* 9(3):255–266
28. Dimitrov KM et al (2019) Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect Genet Evol* 74:103917
29. Babaeimarzangou SS et al (2022) Vaccine development for zoonotic viral diseases caused by positive-sense single-stranded RNA viruses belonging to the Coronaviridae and Togaviridae families. *Experimental Therapeutic Med* 25(1):42
30. Zhao J et al (2017) Efficacy of an inactivated bivalent vaccine against the prevalent strains of Newcastle disease and H9N2 avian influenza. *Virol J* 14:1–8
31. Jamour P et al (2025) Serum reactivity analysis with inactivated GVII-matched vaccine—Payavax G79®: Comparison of B-cell epitopes in NDV-vaccine strains. *Biologicals* 90:101820
32. Iorio RM, Bratt M (1950) Monoclonal antibodies as functional probes of the HN glycoprotein of Newcastle disease virus: antigenic separation of the hemagglutinating and neuraminidase sites. *J Immunol (Baltimore Md : 1984)* 133(4):2215–2219
33. Zhu J et al (2016) Characterization of virulent Newcastle disease viruses from vaccinated chicken flocks in Eastern China. *BMC Vet Res* 12:1–9
34. Sakaguchi T et al (1989) Newcastle disease virus evolution: I. Multiple lineages defined by sequence variability of the hemagglutinin-neuraminidase gene. *Virology* 169(2):260–272
35. Babaeimarzangou SS et al (2023) Molecular characterization and phylogenetic study of the hemagglutinin-neuraminidase gene of newcastle disease virus isolated from peacock (*Pavo cristatus*) and Turkey (*Meleagris*) and its comparison with broiler isolates. *Arch Microbiol* 205(6):253
36. Molouki A et al (2022) Report of a new meq gene size: The first study on genetic characterisation of Marek's disease viruses circulating in Iranian commercial layer and backyard chicken. *Br Poult Sci* 63(2):142–149
37. Tian J et al (2010) Enhanced thermostability of methyl parathion hydrolase from *Ochrobactrum* sp. M231 by rational engineering of a glycine to proline mutation. *FEBS J* 277(23):4901–4908
38. Hu S et al (2009) A vaccine candidate of attenuated genotype VII Newcastle disease virus generated by reverse genetics. *Vaccine* 27(6):904–910
39. Sedeik M et al (2019) Comparative efficacy of commercial inactivated Newcastle disease virus vaccines against Newcastle disease virus genotype VII in broiler chickens. *Poult Sci* 98(5):2000–2007

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