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Serum reactivity analysis with inactivated GVII-matched vaccine—Payavax G79®: Comparison of B-cell epitopes in NDV-vaccine strains

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ABSTRACT

The Newcastle Disease Virus (NDV) sub-genotype VII.1.1 is the most common NDV circulating in Iranian poultry farms. It differs genetically and antigenically from the conventional vaccine strains of genotypes I and II. The inactivated vaccines efficiency can be affected by the grade of similarity with circulating viruses. Here, we updated the NDV vaccine using a local circulating virus seed, introduced Payavax G79® as the inactivated bivalent vaccine, and compared the results with serological and phylogenetic characteristics derived from it to different NDV genotype virus vaccines. According to our results, after 25 days post-vaccination, the antibody titer elicited against sub-genotype VII.1.1 was 8.4 log2. In contrast, the antibody titer against apathogenic genotype I (NDV-V4) and lentogenic genotype II (LaSota) was 4.4 $\log_2$ and 4 log2, respectively. Comparing *in silico* studies of the F protein's discontinued B-cell epitopes, it was found that NDV-GVII, LaSota, and NDV-V4 virus all have seven, four, and eight discontinued B-cell epitopes on the protein's surface. Furthermore, the HN protein surface of NDV-GVII, LaSota, and NDV-V4 virus has four, six, and three discontinued B-cell epitopes, respectively. In summary, the low similarity between NDV genotypes I, II, and the predominant circulating genotype VII (approximately 83–84 %) indicates the need for an updated vaccine seed strain.

1. Introduction

Despite having a single serotype, all Newcastle Disease Virus (NDV) strains exhibit significant differences in their genetic and antigenic properties [1]. This is shown by differences in nucleotide sequences and genome lengths that divide isolates into classes I and II. Class I viruses belong to a single genotype, and three sub-genotypes are typically avirulent in waterfowl and shorebirds. Class II NDV comprises 21 genotypes, designated I to XXI. These viruses have been identified in both pigeons and poultry species. Similarly, the NDV lineages specific to pigeons (genotypes VI and XXI) may have emerged from a lineage adapted to chickens (genotype XX), which historically encompassed chicken-derived viruses [2]. Notably, a genetic distance is observed between modern post-1960 genotypes (V, VI, VII, VIII, and X-XVIII) and ancient pre-1960 genotypes (I, II, III, and IV) within class II, contributing to continued outbreaks among poultry populations [3,4]. NDVs belonging to genotype VII, sub-genotypes VII.1.1 and VII.2, have been identified as the primary agents responsible for fourth- and fifth-generation NDV panzootic [5].

NDV strains are classified into four pathotypes: lentogenic (LaSota and B4), mesogenic (R2B pathogenicity), velogenic (NDV genotype VI), and asymptomatic or avirulent (V4), depending on the severity of the disease they cause in chickens [6,7]. The hemagglutinin-neuraminidase (HN) protein, the cleavage site motifs of the fusion (F) protein, the mean death time (MDT), and the intracerebral pathogenicity index (ICPI) are some of the important parameters that define the virulence of NDV [8].

The NDV envelope has two structural and immunogenic proteins called HN and F. These proteins are very important for determining the virus's tropism, ability to evade neutralizing antibodies, and capacity to infect host [9,10]. The HN protein recognizes and attaches to sialic acid

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receptors present on the surface of susceptible cells. It facilitates the fusion of the F protein at the cellular membrane, allowing the release of the nucleocapsid complex into the cytoplasm. Moreover, phylogenetic analyses can evaluate our understanding of the evolutionary relationships between different genotypes and the relationships between the studied strains [11].

Despite the gradual evolution of NDV into new genotypes over time, the strains utilized in vaccines have remained unchanged since NDV vaccine development in the 1950s. Currently, Newcastle disease poses a considerable threat to the poultry industry each year [12]. Despite the widespread use of various live and inactive vaccines for commercial poultry flocks, outbreaks of the disease are common, exhibiting varying degrees of severity [13]. Although various challenging viruses are accessible globally for vaccine assessment, employing the virus circulating in the local region is deemed the most appropriate approach to manufacture vaccines to elicit immune responses and inhibit virus shedding [14]. A comprehensive review of these studies revealed that the NDV-GVII has prevailed in the nation in recent years [15,16].

Therefore, sub-genotype VII.1.1, the most circulating strain, was used to develop a vaccine against this disease. This study aimed to compare the serum reactivity (analyses of antibody levels elicited by the introduced inactivated) genomic characteristics and amino acid differences, including B-cell epitope mapping and phylogenetic analysis connected to the bivalent vaccine—Payavax G79®—against NDV-GVII compared to other NDV genotypes, such as LaSota and NDV-V4 strains.

2. Materials and methods

2.1. Experimental study

2.1.1. Chickens and vaccination

We conducted this study using a bivalent inactivated vaccine manufactured via Paya Vaccine Tavana Co. [Payavax G79 Batch No. 02-G79-002], which contains 10^8 EID50/dose sub-genotype NDV-GVII and 10^8 EID50/dose H9N2 influenza antigens. The Iranian Administration Committee of Laboratory Animals (IACLA) accredited the animal rearing facilities housing the chickens with approval code AVL. ETH.1402.077.

This experiment was carried out on four groups of two-week-old laying broiler chickens, with 10 chicks in each group housed in separate rooms, receiving the same diet and adhering to breeding guidelines. Three groups were administered a single dose (0.2 mL/chick) of inactivated vaccine Payavax G79, NDV-V4, and LaSota via subcutaneous injection, respectively. The control group included unvaccinated chickens that received Normal saline. The chickens were kept until 35 days post-vaccination. Prior to that, blood samples were collected from each group ($n = 10$) at 25 and 32 days after the vaccination inoculation. The serum was separated and stored at −20 °C for future use.

2.1.2. Hemagglutination-inhibition (HI) assay

The hemagglutination inhibition (HI) assay was employed to determine antibody titers against the NDV-GVII, NDV-V4, and LaSota inactivated vaccines. The OIE-recommended protocol [17], which utilizes 1 % chicken red blood cells, was followed for antibody titer determination.

We collected blood samples from all groups 25 and 32 days after vaccination inoculation. Serum was separated, and complement system inactivation was performed by heat treatment (56 °C for 30 min). Two-fold serial dilutions of the inactivated serum samples were performed in a 96-well plate. A standardized amount of virus (4 HAU) was added to each well of the plate, NDV-GVII virus used for the HI antibody titer test for Payavax G79® vaccinated group, while GII virus and GI virus used for the HI antibody titer test for LaSota and NDV-V4 vaccinated group, respectively. Additionally, the three sub-genotype NDV-GVII, LaSota, and NDV-V4 viruses were added separately to the serum control wells. The plate was incubated at room temperature for 30–60 min to allow for antibody-virus binding. A suspension of red blood cells (RBCs) was then added to each well, and the plate was incubated again at 4 °C for 30 min. The wells were then observed for hemagglutination. We determined the endpoint as the highest serum dilution that showed total inhibition of hemagglutination. HI titers were expressed in log2 units, and samples with titers equal to or greater than 4 log2 were considered positive.

2.2. In silico studies

2.2.1. Genome sequence of different NDV genotypes

The complete genome sequences of NDV-GVII (GenBank accession No. MG867723.1), LaSota (GenBank accession No. AF077761.1), and NDV-V4 (GenBank accession No. JX524203.1) were retrieved from the NCBI gene database and aligned to assess their similarity.

2.2.2. Identification of surface-displaying NDV proteins

The final target proteins were selected based on an evaluation of virulence, subcellular localization, transmembrane topology, and immune protection conferred by neutralizing antibodies. Virulence and envelope proteins were chosen according to the literature. The hemagglutinin-neuraminidase (HN) and fusion (F) proteins on the envelope of the NDV were selected and aligned to determine their similarity across the NDV-GVII, NDV-V4, and LaSota genotypes.

2.2.3. Library of conserved high-score B-cell epitopes

2.2.3.1. Identification of linear B-cell epitopes. The sequences of the HN and F proteins of the three genotypes of NDV were entered into the BepiPred database (http://www.cbs.dtu.dk/services/BepiPred/) with a threshold of ≥0.5. This database employs a sophisticated statistical model called a hidden Markov model to analyze protein sequences. It identifies specific regions within protein sequences that have a high probability of being recognized by immune cells, particularly B cells. These regions are known as continuous B-cell epitopes and play a critical role in the immune response [18].

2.2.3.2. Evaluation of suitable linear B-cell epitopes. Three-dimensional (3D) structural modeling was used to determine surface-exposed linear B-cell epitopes of the HN and F envelope proteins of the three genotypes. Surface-exposed epitopes were determined using Jmol software, version 14.6.4, which is used to determine chemical structures in 3 dimensions (molecular modeling) [19]. Antigenicity was determined with a cutoff ≥0.5 using the VaxiJen webtool (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [20].

2.2.3.3. Identification of discontinuous B-cell epitopes. The 3D structures of the HN and F envelope proteins of the NDV-GVII genotypes, NDV-V4, and LaSota were modeled using the Robetta tool (https://robetta.bakerlab.org/), a server for protein structure prediction. The protein data bank (PDB) ID for NDV-GVII F protein genotypes was 3MAW, NDV-GVII HN protein was 7BWU, LaSota F protein was 1G5G, LaSota HN protein was 3T1E, NDV-V4 F protein was 1G5G and NDV-V4 HN protein was 3T1E. The PDB files generated were utilized to predict discontinuous B-cell epitopes of the HN and F proteins using the ElliPro server (antibody epitope prediction tool), (http://tools.iedb.org/ellipro/). The resulting 3D structures of the surface-exposed discontinuous B-cell epitopes for the HN and F proteins were then obtained and subjected to comparative analysis.

2.3. Phylogeny and genetic distance analysis

We conducted a phylogenetic analysis to compare the genetic relationships among various Newcastle Disease Virus strains, such as NDV-GVII (Payavax G79®), NDV-V4 (genotype I), and LaSota (genotype II). The study retrieved complete genome sequences from the NCBI

database, aligned them using the ClustalW algorithm (http://www.megasoftware.net), and constructed a phylogenetic tree to assess vaccine strains' evolutionary divergence and relatedness. We created a phylogenetic tree using the maximum likelihood method, where branch lengths, with values, represented the genetic distance between strains.

2.4. Statistical analysis

In this study, GraphPad Prism 9 was used for data analysis, and ANOVA and descriptive statistical tests were used. A significance level of $p \leq 0.05$ was applied for all tests.

3. Results

3.1. Serological responses

In this experiment, we compared the results of the HI assay in vaccination groups, including Payavax G79® against NDV-GVII, NDV V4, LaSota, and control. Generally, the control group did not show detectable specific antibodies at either 25- or 32-days post-vaccination. The immune responses post-vaccination with Payavax G79® were evaluated, and the mean HI titers demonstrated that Payavax G79® elicited a superior antibody response with a mean HI antibody titer of 8.4 log2 on day 25 and 9.5 log2 on day 32 ($p < 0.05$). In contrast, the LaSota vaccine group showed the lowest titers, 4 log2 on day 25 and 4.5 log2 on day 32 ($p < 0.05$). The results for the NDV-V4 vaccine group were approximately similar to those for the LaSota vaccine group, with mean HI titers of 4.4 log2 on day 25 and 5 log2 on day 32. Therefore, the mean HI titers in the Payavax G79® group were higher than the other groups at 25- and 32-day post-vaccination (Fig. 1).

3.2. Comparative sequence analysis of complete genomes and HN/F proteins in NDV genotypes

Alignments of the complete genome sequences of various NDV genotypes revealed differences in nucleotide composition. Specifically, NDV-GVII had a length of 15,192 nucleotides, whereas NDV-V4 and LaSota had lengths of 15,186 nucleotides (Supplementary Data 1). Comparative analysis revealed nucleotide disparities of 2285 between NDV-GVII and NDV- V4, 2580 between NDV-GVII and LaSota, and 1587 between NDV-V4 and LaSota (Fig. 2). The similarity percentages were 84.96 % between NDV-GVII and NDV-V4, 83.02 % between NDV-GVII and LaSota, and 89.55 % between NDV-V4 and LaSota (Fig. 2A–Supplementary Data 2). These findings suggest that NDV-GVII, compared with the two NDV genotypes, exhibit the most variation in nucleotide count and the least similarity percentage with NDV-V4 and LaSota. This indicates its potential uniqueness in stimulating the immune system and in vaccine development.

Furthermore, a comparative analysis of the F protein sequences revealed differences of 53 amino acids between NDV-GVII and NDV-V4, 65 amino acids between NDV-GVII and LaSota, and 38 amino acids between NDV-V4 and LaSota. The similarity percentages were 90.42 % between NDV-GVII and NDV-V4, 88.25 % between NDV-GVII and LaSota, and 93.13 % between NDV-V4 and LaSota (Fig. 2B–Supplementary Data 3). These results indicate that the highest similarity percentage exists between NDV-V4 and LaSota, while NDV-GVII exhibits notable differences compared to both.

Similarly, comparison of the HN protein sequences revealed differences of 96 amino acids between NDV-GVII and NDV-V4, 67 amino acids between NDV-GVII and LaSota, and 70 amino acids between NDV-V4 and LaSota. The similarity percentages were 84.42 % between NDV-GVII and NDV-V4, 88.39 % between NDV-GVII and LaSota, and 88.64 % between NDV-V4 and LaSota (Fig. 2C–Supplementary Data 3). These

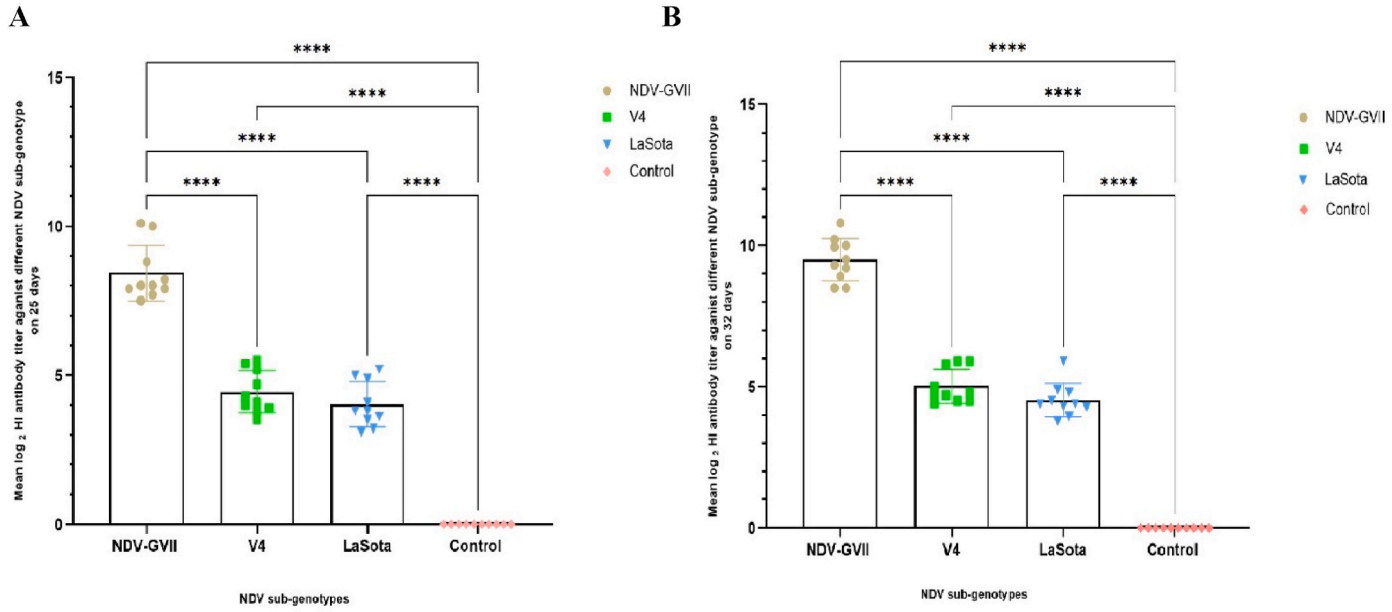

Fig. 1. The mean HI antibody titer in different vaccination groups. The groups of chickens were vaccinated with different NDV sub-genotype vaccines, including Payavax G79® against NDV-GVII, NDV-V4, LaSota, and control group. In the control group, chickens received normal saline without any NDV sub-genotype vaccines. Blood samples were collected equally from all groups. For determination of HI antibody titers, NDV-GVII virus was used for the Payavax G79® vaccinated group, the GI virus for the NDV-V4 vaccinated group, and the GII virus was used for the Lasota vaccinated group. Furthermore, each of the three viral sub-genotypes was individually tested on the control group serum. Since the HI antibody titer test results for all three were similar, they were presented as a single column. Each data point represents an individual chicken from the experimental groups. In part A (left), the comparison of the mean HI titer (log 2 ± SD) at 25 days post-vaccination is shown for different vaccination groups with Payavax G79® against NDV-GVII, NDV-V4, LaSota, and control, and in part B (right) shows the comparison of the mean HI titer at 32 days post-vaccination in various vaccination groups including Payavax G79® against NDV-GVII, NDV-V4, LaSota, and control. Statistical analysis was conducted using one-way analysis of variance (ANOVA), and significant differences between groups were indicated by $p < 0.05$. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

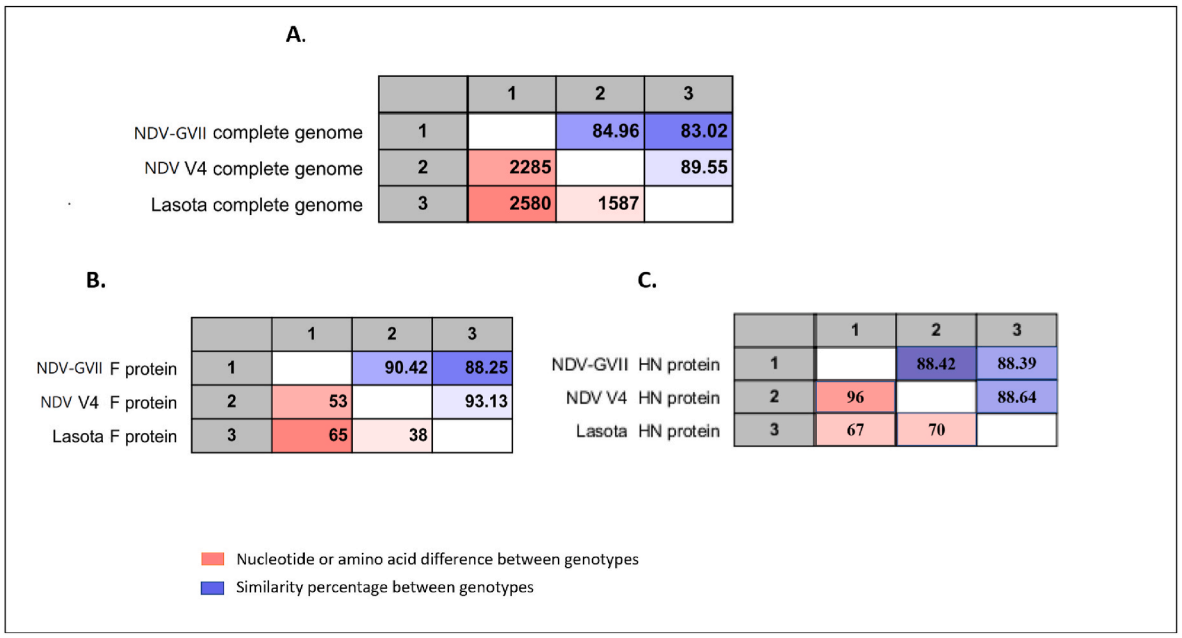

Fig. 2. The nucleotide or amino acid sequences of the different NDV genotypes were compared. **A**, Comparison of the complete genomes of NDV-GVII, LaSota and NDV-V4. The results revealed a difference of 2285 nucleotides between NDV-GVII and NDV-V4, a difference of 2580 nucleotides between NDV-GVII and LaSota and a difference of 1587 nucleotides between NDV-V4 and LaSota. The percentage of similarity between NDV-GVII and NDV-V4 was 84.96 %, that between NDV-GVII and LaSota was 83.02 %, and that between NDV-V4 and LaSota was 89.55 %. **B**, Comparison of the F protein sequences of NDV-GVII, LaSota and NDV V4. The results revealed 53-amino acid difference between NDV-GVII and NDV-V4, 65-amino acid difference between NDV-GVII and LaSota and 38-amino acid difference between NDV-V4 and LaSota. The percentage of similarity between NDV-GVII and NDV-V4 was 90.42 %, that between NDV-GVII and LaSota was 88.25 %, and that between NDV-V4 and LaSota was 93.13 %. **C**, Comparison of the HN protein sequences of NDV-GVII, LaSota and NDV V4. The results revealed 96-amino acid difference between NDV-GVII and NDV-V4, 67-amino acid difference between NDV-GVII and LaSota and 70-amino acid difference between NDV-V4 and LaSota. The percentage of similarity between NDV-GVII and NDV-V4 was 84.42 %, that between NDV-GVII and LaSota was 88.39 %, and that between NDV-V4 and LaSota was 88.64 %.

findings also highlight that NDV-V4 and LaSota exhibit the greatest similarity percentage, while NDV-GVII demonstrates significant differences when compared to both of these genotypes.

3.3. Prediction of linear B-cell epitopes and high-scoring antigen library construction for diverse NDV genotypes

Epitopes from the F and HN proteins of the NDV-GVII, NDV V4, and LaSota genotypes were identified using the BepiPred database. The NDV-GVII F protein exhibited 16 linear epitopes, 8 of which had antigenicity scores ≥0.5, while its HN protein had 15 linear epitopes, with 9 high-scoring antigen epitopes. The F protein of NDV-V4 has 13 linear epitopes, with 6 scoring ≥0.5, and its HN protein has 16 linear epitopes, 10 of which are high-scoring antigens. LaSota's F protein displayed 15 linear epitopes, 5 with scores ≥0.5, and its HN protein had 16 linear epitopes and 10 high-scoring antigens (Supplementary Data 4). Comparative analysis revealed that the F protein epitopes of three subgenotype NDV, such as NDV-GVII, LaSota, and NDV-V4, were more similar to the HN protein. Additionally, NDV-V4 and LaSota exhibited similar high-scoring epitopes in the HN protein (Supplementary Data 4). Additionally, visualization of antigen epitopes in the 3D structure of the F and HN proteins using Jmol software was conducted (Fig. 3).

3.4. Prediction of discontinuous B-cell epitopes of different NDV genotypes

The 3D conformations of the F and HN proteins belonging to the NDV-GVII, NDV-V4, and LaSota genotypes were predicted utilizing the Robetta web server (Figs. 4 and 5). Discontinuous B-cell epitopes were subsequently forecasted through employment of the Ellipro server and subsequently mapped onto the respective 3D structures of each protein (Supplementary Data 5). The results revealed that NDV-GVII harbors 7 discontinuous B-cell epitopes situated on the surface of the F protein and that LaSota possesses 4 such epitopes, while NDV-V4 exhibits 8 discontinuous B-cell epitopes (Fig. 4). Furthermore, the investigation revealed that NDV-GVII has 4 discontinuous B-cell epitopes on the surface of the HN protein, LaSota has 6, and NDV-V4 has 3 discontinuous B-cell epitopes (Fig. 5).

3.5. Phylogenetic analysis of NDV strains used in Payavax g79® and other NDV vaccines

We used phylogenetic analysis to examine the genetic relationships between the NDV strains used in the Payavax G79® vaccine (genotype VII.1.1) and the conventional vaccine strains, LaSota (genotype II) and NDV-V4 (genotype I). The following phylogenetic tree, which represents genetic distances by numerical values on the branches, depicts the evolutionary divergence between these strains (Fig. 6, Supplementary Data 6). Fig. 6 demonstrated the significant genetic diversity between NDV-GVII and prior vaccine strains, revealing a genetic distance of 6 % variation in the nucleotide sequence, 5 % variation for the *F* gene, and 7 % variation for the *HN* gene. This highlights the potential of genotype-updated vaccines, including Payavax G79®, to successfully protect against strains of NDV that are circulating in chicken farms.

4. Discussion

Despite extensive vaccination programs, both vaccinated and non-vaccinated poultry flocks are consistently affected by ND epidemics. The outer surface proteins of NDV, particularly the F and HN proteins, play crucial roles in viral virulence, tropism, and immune protection through neutralizing antibodies [21]. The divergence in neutralizing epitopes between vaccine strains and circulating pathogenic viruses might contribute to the insufficient efficacy of these vaccines. To enhance

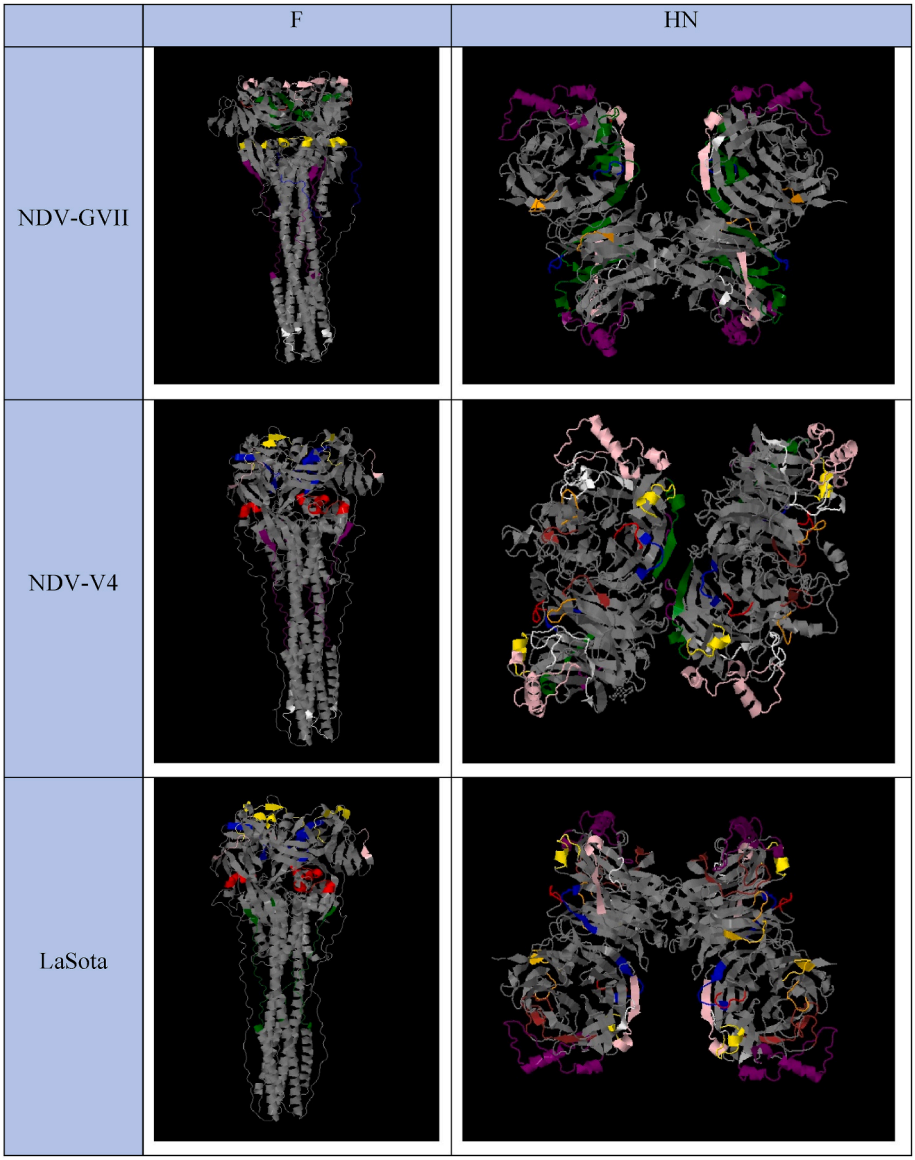

Fig. 3. Determination of the localization of linear B-cell epitopes on the tertiary structures of the F and HN proteins of the NDV-GVII, LaSota and NDV V4 genotypes using Jmol software. The antigenic epitopes are colorful due to surface exposure of the 3D structure of the protein.

immunization effectiveness in terms of reducing infectivity and minimizing virus shedding, a greater percentage of amino acid similarity in F and HN proteins between NDV strains and field viral vaccines is necessary [22].

The current study carried out an *in silico* and experimental analysis of the immunological properties of an NDV-GVII-matched vaccine with other strains of the NDV virus. Previous studies have demonstrated high protection and reduced virus shedding in chickens immunized with recombinant vaccines, particularly those targeting genotype I or the heterologous genotypes VII.1.1, VII.2, and V of NDV [23]. Hence, this study aimed to compare the HI titer of the three strains post-vaccination with the vaccine. Additionally, the B-cell epitopes of the F and HN proteins found in the NDV-GVII, LaSota, and NDV-V4 virus strains were compared, emphasizing the importance of increased genetic and antigenic matching of F and HN proteins for enhanced protections [12].

In this study, at 32 days post-vaccination, the highest mean HI antibody titer for the NDV-GVII strain was observed, indicating a significant post-vaccination antibody response [24]. Miller et al. [25] demonstrated that variations in neutralizing epitopes between circulating viruses and vaccine strains may contribute to the observed differences in vaccine efficacy. Additionally, *F* gene mutations in circulating viruses might influence vaccine effectiveness. HI titers reflect several factors, including disease incidence reduction, antibody production level, viral shedding reduction post-vaccination, and decreased mortality rates [26]. Serological tests revealed that amino acid changes can impact antigenic variation among NDV genotypes I, II, VII. Unsurprisingly, closely genotype-matched vaccines provide better protection against mortality than non-genotype-matched vaccines [27]. Studies consistently show that vaccines closely matched to circulating genotypes provide superior protection against both morbidity and mortality compared to non-genotype-matched vaccines [1,28].

In silico studies comparing different NDV genotypes revealed distinct nucleic acid sequence lengths and differences in HN and F protein similarity. Antigenic epitope analysis indicated that, compared with other genotypes, NDV-GVII exhibited suitable linear and discontinuous epitopes on the HN and F proteins, potentially contributing to its superior antibody response [29,30]. Thus, the favorable results of the NDV-GVII HI antibody titer may be attributed to its epitopic characteristics and accessibility. Different F and HN protein epitopes affect the levels of HI after vaccination in different NDV genotypes [9,31].

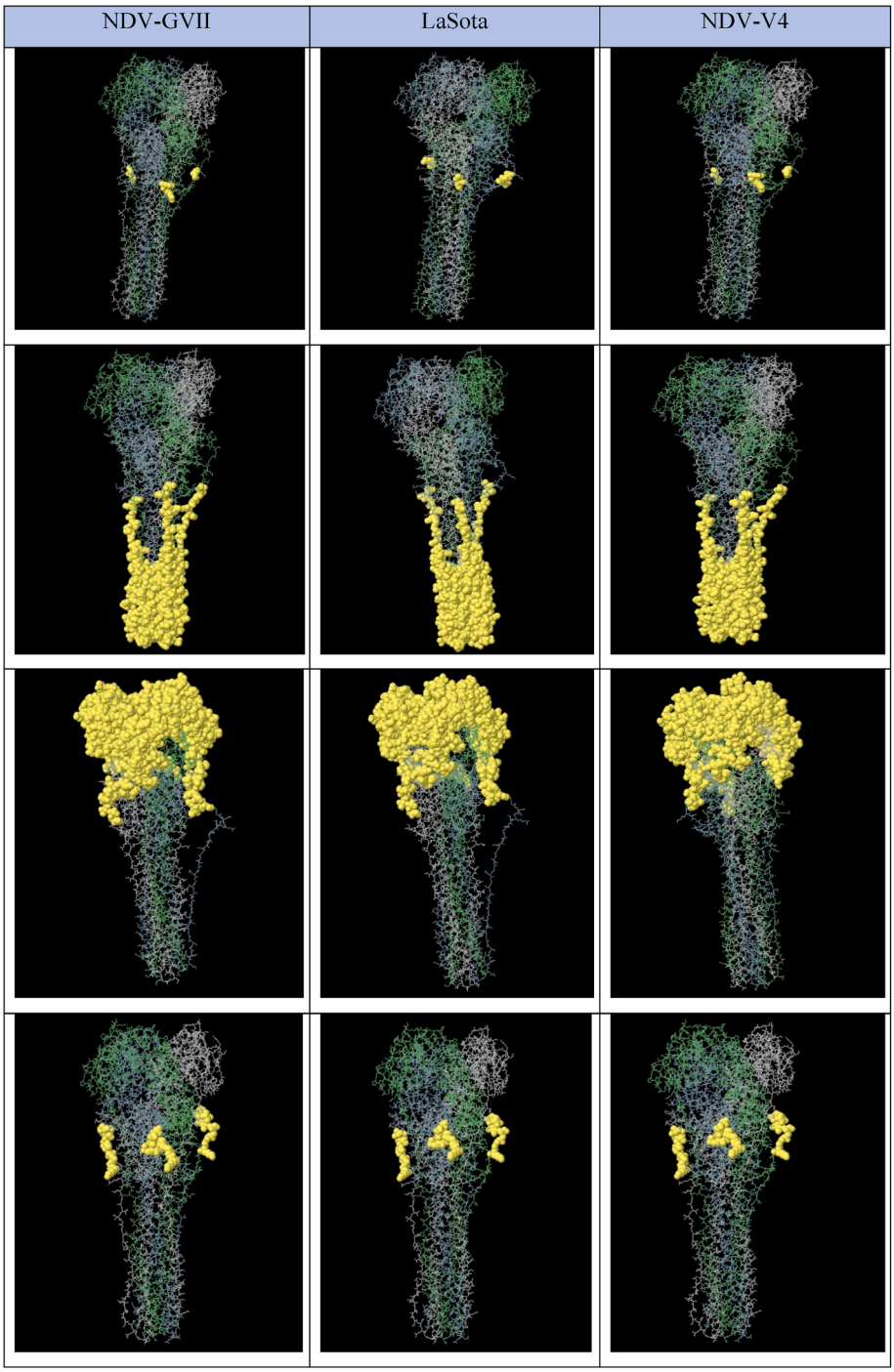

Fig. 4. Comparison of 3D structures of F protein discontinuous B-cell epitopes in NDV-GVII, LaSota and NDV-V4 genotypes. The results indicate that NDV-GVII has 7 discontinued B-cell epitopes on the surface of the F protein, LaSota has 4 discontinued B-cell epitopes, and NDV-V4 has 8 discontinued B-cell epitopes. Yellow balls indicate discontinued B-cell epitopes on the surface of the F protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Hosseini et al. [32] demonstrated that high-ranking epitopes, such as MRATYLETL and LYCTRIVTF, have strong binding affinities to MHC class I alleles, which may induce robust immunological responses. The study has shown that the genetic diversity of the F gene influences the antigenic variation, as mutations can modify immune recognition and effectiveness of the epitopes on this protein. It seems that genotype-specific vaccines, like NDV-GVII, may offer better protection because they contain high-affinity epitopes and overlap between MHC class I, II, and B-cell epitopes. We found antigenic conformational and linear B-cell epitopes during our study. This may help explain why the NDV-GVII strain is more likely to cause an immune response. The presence of distinct nucleic acid sequences in the F and HN proteins, among other genotypes, likely caused this difference. Moreover, our study emphasizes the significance of considering both linear and conformational epitopes in vaccine design, in line with other studies. The NDV-GVII strain is a great choice for more vaccine studies because its HN and F proteins are full of epitopes, which may help make it more immunogenic [32–34].

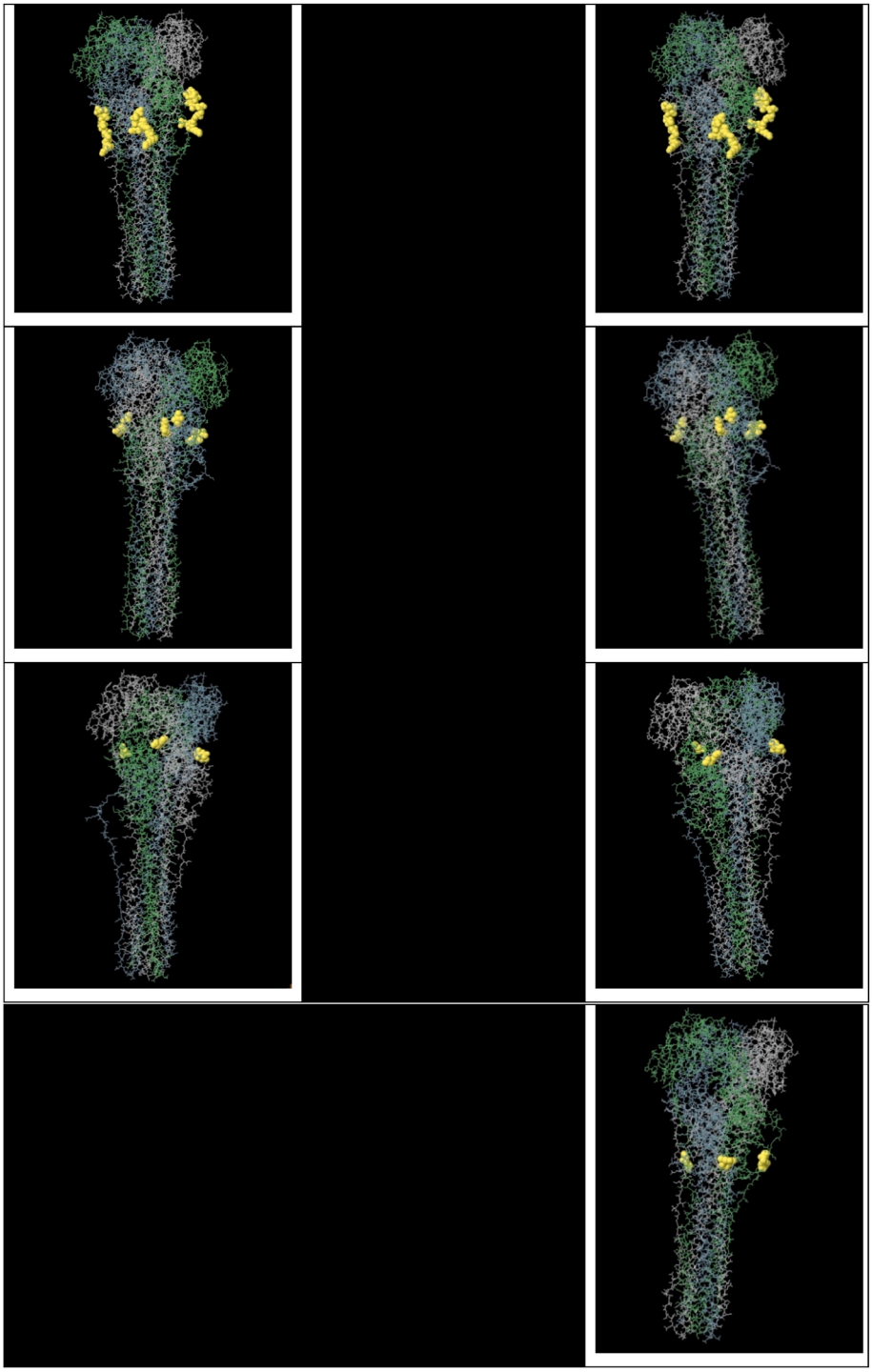

Fig. 4. (*continued*).

Based on our phylogenetic tree, NDV strains, especially NDV-GVII (Payavax G79®), are genetically very different from other vaccine strains. There are 6 % differences in the whole genome, 5 % differences in the *F* gene, and 7 % differences in the *HN* gene. The F and HN proteins have changed a lot during the NDV-GVII strain's unique evolutionary process, as shown by these differences. Because genetic variations can reduce the efficacy of current vaccines, genotype-matched vaccines are necessary to provide the best protection against emerging NDV strains. These findings were consistent with previous studies [7].

The F and HN genes encode important viral proteins with effective epitopes essential for eliciting the neutralizing antibodies. These effective epitopes, especially those found inside neutralizing regions, determine the effectiveness of the vaccination in providing protection. Minor mismatches between the vaccine strains and circulating strains at these epitopes may result in partial protection, but a complete match ensures optimal protection. These areas are highlighted by epitope mapping, which also helps to explain the variations in immune responses seen between strains, particularly with regard to the F and HN proteins. Our findings are consistent with previous studies that have demonstrated the importance of sequence matching and epitope positioning in vaccine efficacy [25,35–37].

An important factor affecting the efficacy of inactivated NDV

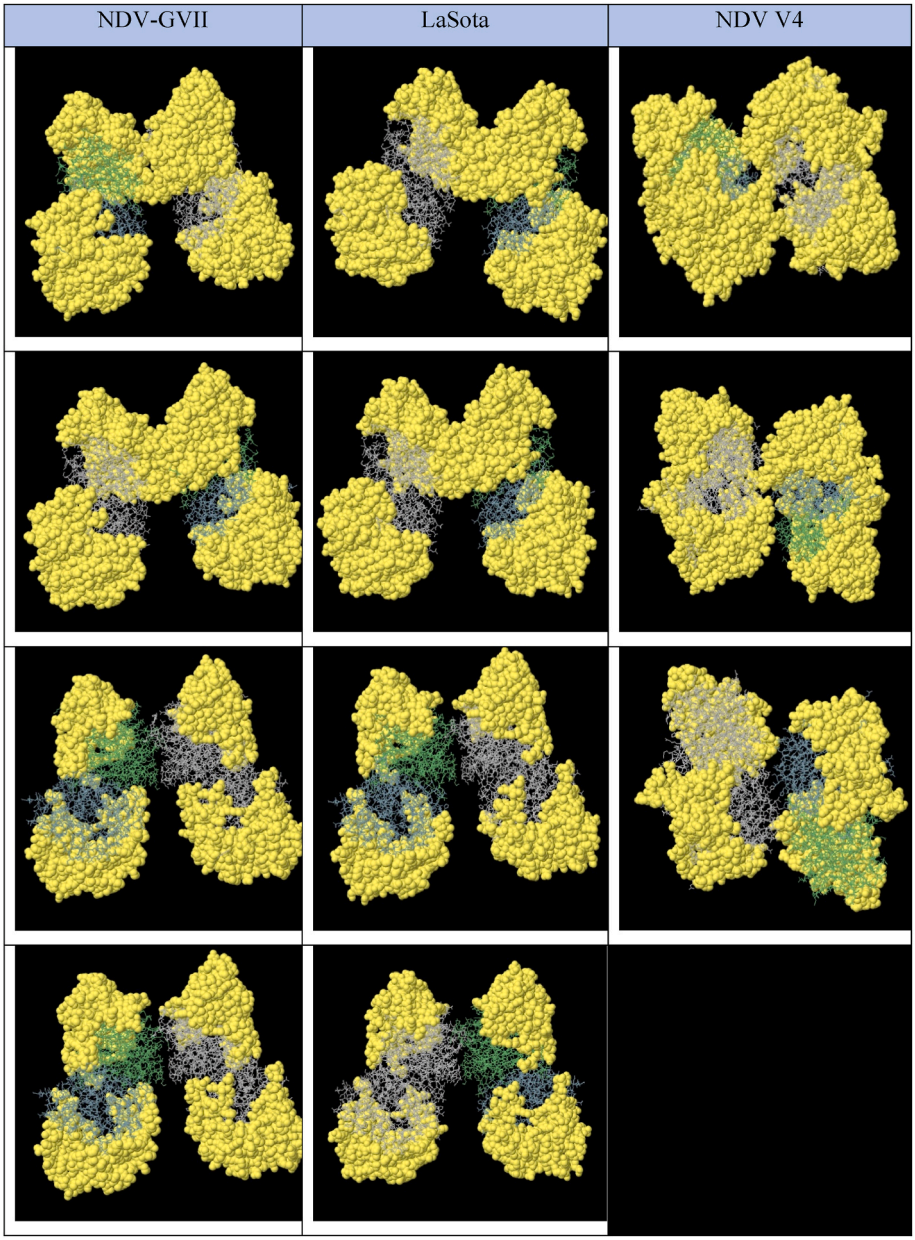

Fig. 5. Comparison of 3D structures of HN envelope protein discontinuous B-cell epitopes in NDV-GVII, LaSota and NDV-V4 genotypes. The results indicate that NDV-GVII has 4 discontinued B-cell epitopes on the surface of the HN protein, LaSota has 6 discontinued B-cell epitopes, and NDV-V4 has 3 discontinued B-cell epitopes. Yellow balls indicate discontinued B-cell epitopes on the surface of the HN protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

vaccines is their antigenic match to circulating virus strains. The protection against systemic diseases caused via velogenic pathotype offered by homologous inactivated vaccinations is superior. Whether injected subcutaneously or intramuscularly, inactivated vaccines can stimulate a robust and sustained immune response, successfully protecting against systemic infections. The result of recent study suggests that, with amino acid identities for the F and HN proteins of 87–89 % and 87–88 %, respectively, there is a significant genetic difference between predominant NDV strains and conventional vaccination strains. LaSota-based vaccinations, as opposed to homologous ones, result in decreased hemagglutination inhibition titers against heterologous viruses as a result. Although traditional vaccinations protect against diseases and fatalities, immunized hens are nevertheless capable of shedding a significant amount of the virus, but add to the regular incidence of unusual Newcastle disease in flocks [10,25]. We suggest that, in contrast to the LaSota vaccination, genotype-matched vaccines as an inactivated GVII-matched vaccine, can elicit a stronger and faster antibody response, providing complete protection against genotype VII virus challenges in hens and significantly lowering virus shedding. Unlike respiratory route administration of live attenuated vaccine, these injectable and homolog and matched vaccines can induce substantial humoral immunity, which makes them particularly effective in preventing systemic diseases [38].

Finally, the quantity of discontinuous B-cell epitopes does not indicate their relevance in vaccination immunogenicity. Better viral neutralization may be made possible by the NDV-GVII vaccine's targeted selection of highly immunogenic B-cell epitopes and the strong antibody response noticed by greater HI titers [39]. The HI titer, a

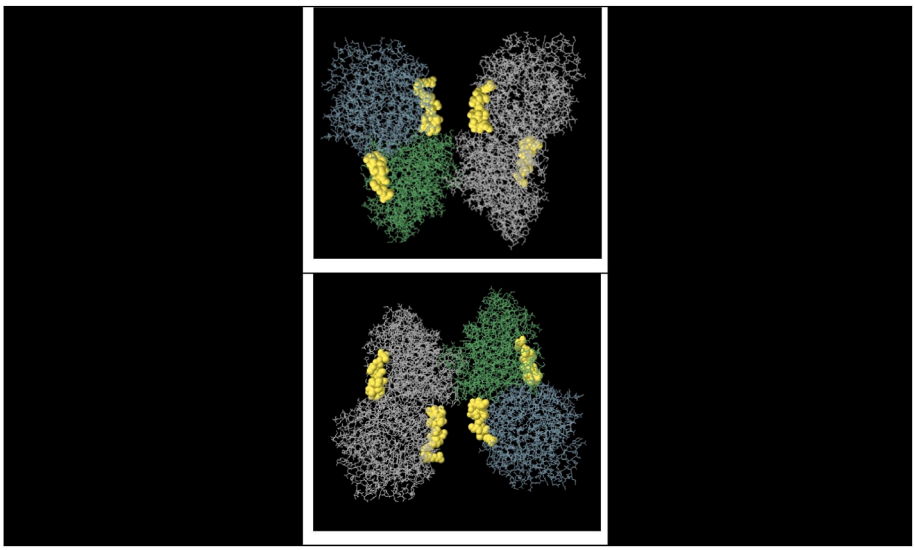

Fig. 5. (*continued*).

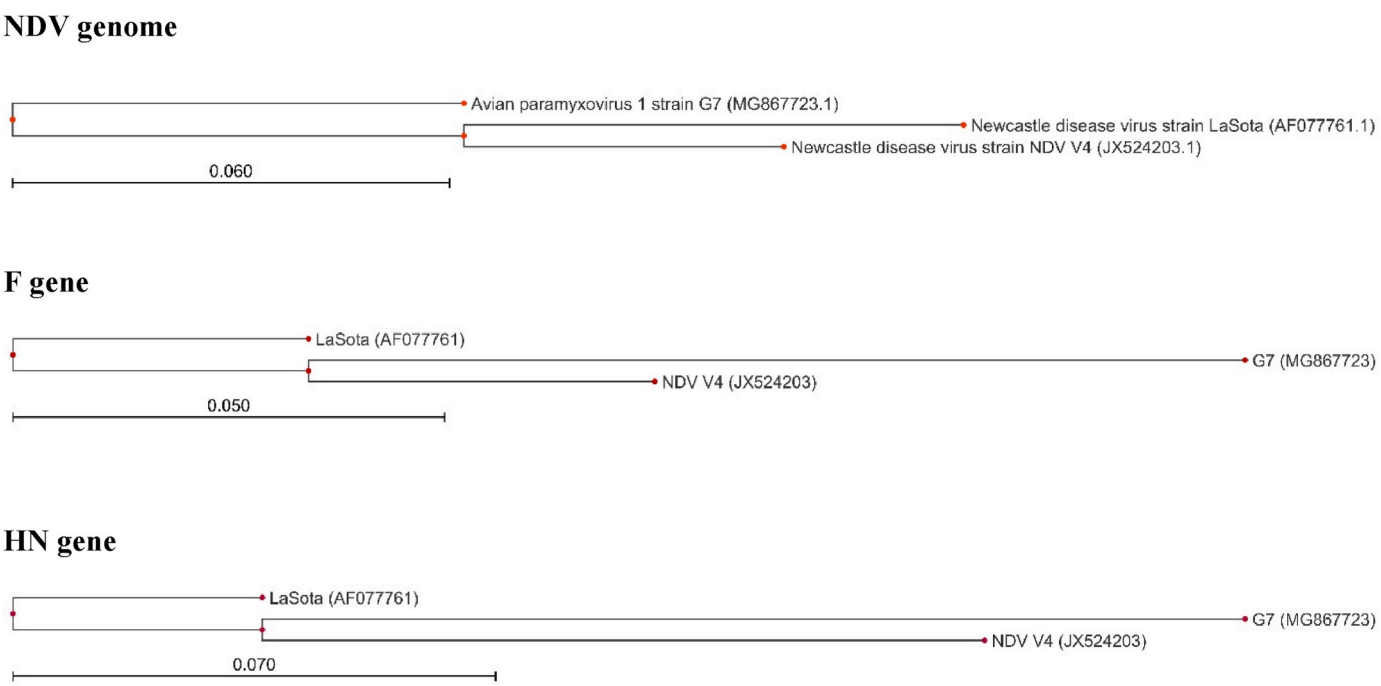

Fig. 6. Phylogenetic analysis of NDV strains. The phylogenetic tree of NDV strains showed that NDV-GVII (Payavax G79®) is genetically distant from other vaccine strains by 0.060 for the full genome, 0.050 for the F gene, and 0.070 for the HN gene. These values indicate a 6 % divergence in the complete genome, which means that divergence highlights the distinct evolutionary path of the NDV-GVII strain. The F protein's amino acid sequences differ by 5 %. Since the F protein is very important for both entering and neutralizing viruses, the observed differences may affect how well vaccines work to protect against them. Given how important the HN protein is for binding receptors and immune recognition, a 7 % difference in it could make it harder for current vaccines to work against the NDV-GVII strain. It highlights significant genetic variation, emphasizing the need for updated genotype-matched vaccines that align closely with currently circulating strains to ensure effective protection against circulating NDV strains.

measure of the effectiveness of the immune response, correlates with protection. The NDV-GVII vaccine may be more immunogenic due to its fewer but more durable and accessible discontinuous B-cell epitopes [40]. There may be fewer but structurally intact discontinuous B-cell epitopes in the NDV-GVII vaccine, which may improve immunodominance and lower competition for immunological resources. This could lead to a stronger antibody response and higher HI titers. Furthermore, the vaccine strain affects the immunological response and epitope significance [41]. Therefore, while a higher number of discontinuous B-cell epitopes might seem advantageous, the quality and functionality of these epitopes are paramount. A vaccine with fewer but more immunogenic and functionally relevant epitopes can elicit a stronger antibody response and potentially offer better protection, as evidenced by the higher HI titer.

Some of the study's limitations include the focus on specific NDV genotypes such as NDV-GVII, LaSota, and NDV-V4 in comparison to other genotypes, the limited sample size of chickens in each experimental group, and the use of *in vitro* analysis, which could not accurately represent *in vivo* immune responses.

5. Conclusion

The serum reactivity of the NDV-GVII-matched vaccine elicited a strong antibody response that specifically targeted velogenic NDV-GVII. This response was stronger than the responses seen with NDV genotypes II and I. Furthermore, there are significant genetic and antigenic differences between conventional vaccination strains and the predominant genotype VII, which could potentially limit the effectiveness of currently used vaccines. This highlights the vital need to update vaccine strains to better match the circulating genotypes. The GVII-based vaccine improved efficacy in chickens by providing superior protection and reducing viral shedding.

We suggest that genotype-matched vaccinations could significantly improve disease management and curb the spread of NDV in poultry farms. This shows the significance of genotype-specific vaccines in enhancing NDV control strategies.

CRediT authorship contribution statement

Parisa Jamour: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Maryam Shafaati:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Mostafa Gholizadeh Gigloo:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Rasa Sheini Mehrabzadeh:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Towhid Mohammadi:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Majid Lotfinia:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Sanaz Majidi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

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Declaration of competing interest

Ms. Sanaz Majidi is the Technical Manager at Paya Vaccine Tavana Company. The other authors declare that they have no competing interests.

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Appendix A. Supplementary data

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