

The Predominance of Newcastle Disease Virus Genotype VII: Genome Diversity or Poor Cross-Immunity of non-Matched Vaccines

Shahla Shahsavandi*, Mohammad Majid Ebrahimi, Majid Tebianian

Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran

ARTICLE INFO

Research Article

VacRes, 2021

Vol. 8, No.2, 4- 16

Received: February 23, 2022

Accepted: March 06, 2022

Pasteur Institute of Iran

*Corresponding Author:

Shahla Shahsavandi

Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran

Email: s.shahsavandi@rvsri.ac.ir

Tel/Fax: +982634570038-46

/+982634552194

KEYWORDS: Newcastle disease virus, vaccine, genetic divergence, immune responses

ABSTRACT

Introduction: The virulent Newcastle disease virus (NDV) strains cause an economically important infectious disease in poultry. The common vaccination program with genotype II NDV strains is routinely practiced to provide a better protection level against Newcastle disease (ND). Nevertheless, the emergence of new antigenic and genetic variants within the circulating NDVs raises the importance of improved control strategies. The genotype VII NDV is associated with many of the most recent outbreaks of the disease worldwide. **Methods:** We evaluated the impacts of genetic divergence between the genotypes II and VII on the immunity against NDV to choose a suitable vaccine virus candidate by focusing on the F and HN proteins. Comparative bioinformatics analyses based on B- and T-cell epitopes binding affinity, protein secondary structure and physicochemical properties predictions were applied for genotypes II and VII. **Results:** Although the results showed more differences in HN protein than F protein, there was no major difference between the predicted antigenicity values, epitope regions, affinity binding to MHC-I and MHC-II, secondary structures, surface accessibility, and stability of these immunogens between genotypes II and VII. **Conclusion:** The results suggest that genotype II-based live vaccines can induce immune responses against NDV; however, an inactivated vaccine formulated by genotype VII should be considered in combination with the traditional live vaccine to provide better protection in controlling programs against ND.

Citation:

INTRODUCTION

Newcastle disease (ND) is one of the most important avian infectious diseases which significantly affects poultry production in the world. The disease is caused by ND virus (NDV), belonging to Avulavirus genus of Paramyxoviridae family that is classified into 9 genotypes in class I and 18 (I to XVIII) genotypes in class II [1]. Since 1926, four NDV panzootic outbreaks have been defined, resulting in massive economic losses to the poultry industry [2]. The NDV single-stranded and negative-sense RNA genome encodes four structural proteins, namely, nucleoprotein, matrix protein, fusion and hemagglutinin-neuraminidase glycoproteins, and two non-structural proteins which are large polymerase protein and phosphoprotein in 3'-NP-P-M-F-HN-L-5' order [3]. The non-structural proteins, V and W, are also produced by RNA editing during P gene transcription [4]. The HN is a type II homotetrameric membrane protein consisting of an N-terminal transmembrane domain and an ectodomain responsible for receptor binding via sialic acid, neuraminidase activity, and membrane fusion through interaction with the F protein.

Thereby, the glycoprotein involves in the entry of viral RNA and subsequent release of progenies from the infected

cells including the respiratory, nervous, and digestive systems, and therefore, determining the tissue tropisms of NDV [5, 6]. The F glycoprotein is a class I transmembrane protein that mediates the virus-host cell membrane fusion. The motif at positions 112-117 of the proteolytic cleavage site in F0 precursor protein is relevant to systemic replication and virulence of NDVs. Along with these functions, stimulation of virus-neutralizing antibody production is the critical action of these proteins [7, 8].

The ND vaccination is routinely practiced in countries where virulent strains of NDV are endemic to prevent significant economic consequences for the poultry industry. The mass vaccinations against NDV have effectively suppressed the global outbreak of the disease. The current NDV live vaccines composed of B1 Hitchner, LaSota, and Clone belong to genotype II and induce acceptable immunity levels against heterogeneous viruses. However, ND is still one of the most important avian diseases and frequent outbreaks or sporadic cases of NDV, and virus shedding are being reported with high losses among the infected flocks in many countries [9]. Genotype VII NDV strains emerged in the 1990s and are

now predominant in Asia and the Middle East, Europe, South Africa, and South America [10-13]. The repeated ND outbreaks despite immunization may indicate the ineffectiveness of the current vaccines due to antigenic divergence between the vaccine strain and the circulating field strains as well as vaccination practices in controlling the disease. In this regard, the development of genotype-matched vaccines to control the new generation or multiple genotypes has been considered. Reverse genetic and vector-based co-expression of NDV F or HN protein techniques are the main vaccine production strategies that have received more attention in recent years [14-17].

Although these vaccines induce antibody responses, their production and/or farm administration are limited. Despite some ND outbreaks, most global vaccine manufacturers apply egg-based technology, using the genotype II viruses for the large-scale production of NDV vaccines. The objective of this study was to inquire whether the genetic diversity between the genotypes II and VII as the predominant field strain of NDV may reduce the efficacy of the current vaccines. Considering the role of F and HN proteins in inducing the immune responses against NDV, we focused on the differences between B- and T-cell epitopes binding affinities, protein secondary structure, and physicochemical properties of LaSota (a commercial vaccine strain) and genotype VII. This approach is significant for selecting the proper vaccine strain to improve the immunization against ND in the controlling programs.

MATERIALS AND METHODS

F and HN Protein Sequences Retrieval

The deposited F and HN sequences of NDVs belonging to genotype VII were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/protein>) and were subjected to multiple sequence alignments using ClustalW (BioEdit software version 7.2.5). We chose F and HN sequences of the genotype (accession numbers JQ267579 and KU938925) regardless of their sub-genotype. The sequences of LaSota strain (accession numbers KU665482 and KU665483) were selected for comparative analysis. The potential of these proteins for induction of humoral and cell-mediated immune responses through B- and T-cells epitope predictions were analyzed using B- and T-cell epitopes prediction tools at the Immune Epitope Database analysis resource (<http://www.iedb.org/>) [18].

B-Cell, T-Cell and MHC Epitope

The B cell epitopes were predicted by Bepipred Linear Epitope Prediction 2.0 with a default threshold of 0.50. The antigenicity and the antigenic sites were identified using Kolaskar and Tongaonker algorithm. The specific T-cell responses based on major histocompatibility complex (MHC)-presented peptides were predicted by TepiTool available in IEDB. The binding affinity of cytotoxic T lymphocyte (CTL) epitopes binding to MHC class I was predicted by the Artificial Neural Network (ANN) algorithm using the panel of most frequent A and B HLA alleles. The IC₅₀ cutoff for peptide selection criterion was set <50 nM to predict the strong 9-mer MHC-I binders. The promiscuity based on the 7-allele method was selected for MHC-II binding helper T lymphocyte (HTL) epitopes prediction. Median consensus percentile ≤ 20 was considered a strong binding affinity.

Predictions of Transmembrane Helices, Physicochemical Properties and Protein Secondary Structures

Differences in the genomic content of sequences may affect the features or functions of the proteins. The effect was monitored by the prediction of secondary structures of LaSota F and HN proteins compared with genotype VII using PSIPRED available at (<http://bioinf.cs.ucl.ac.uk/psipred/>) [19]. The number of transmembrane helices that are predominantly composed of hydrophobic residues was predicted using TMHMM Server (<http://www.cbs.dtu.dk/services/TMHMM/>) [20]. The physicochemical parameters included theoretical isoelectric point (pI), negatively and positively charges, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) were determined using the ProtParam (<http://web.expasy.org/protparam/>) [21]

RESULTS

The Prediction of B-Cell Epitopes

The predicted linear B-cell epitopes of F and HN proteins results are summarized in Tables 1 and 2, respectively. With slight differences, the positions and epitope sequences of F protein were the same in both genotypes. These differences were found in positions 364 and 482 in LaSota strain and in position 151 in genotype VII virus (Table 1). In HN protein, the prediction of B lymphocyte binding affinity showed more differences between the two genotypes, especially at the beginning of the sequence (Table 2). Kolaskar and Tongaonkar's algorithm estimated the average antigenicity 1.048 and 1.043 for F protein of LaSota and genotype VII, respectively, as well as 1.205 and 1.036 for HN protein, where the threshold value > 1.00 was potentiality antigenic.

The Prediction of T-Cell Epitopes

The MHC-I binding prediction results of F protein T cell epitopes strongly linked (IC₅₀ value <50 nM) with the most frequent HLA-A and -B alleles are set out in Table 3. Many MHC-I epitopes were predicted which could activate CTLs. The relatively similar prediction data were achieved for the both genotypes. Most differences were observed in positions 421 to 512; therefore, either the epitope was not identified or one epitope was interacting and linked with more alleles.

The MHC-II binding predictions of the protein-based on 7-allele method are summarized in Table 4. The median consensus percentile for the peptide selection was < 20. As shown in this Table, the number of the HTL epitopes linked to the alleles was higher in genotype VII than in genotype II. The binding affinity was also greater in similar epitopic positions. Four epitopes residues of F protein from MHC-I at positions 116, 381, 491, and 501 were overlapped with MHC-II epitopes in genotype VII. However, the overlapping was not observed between MHC-I and -II of the LaSota strain.

Table 1. The B cell epitope prediction of NDV fusion protein.

LaSota			Genotype VII		
Start	End	Peptide	Start	End	Peptide
5	10	PSTKNP	5	8	PSTR
30	35	NSIDGR	31	35	SLDGR
54	58	TSSQT	54	57	TSSQ
68	86	NLPKDKEACAKAPLDAYNR	68	86	NMPKDKEACAKAPLEAYNR
100	114	RRIQESVTTSGGGRQ	103	114	QGSVSTSGGRRR
149	152	ANIL	149	149	A
NO	NO	NO	151	151	I
188	189	DQ	187	189	NDQ
191	193	NKT	191	193	NNT
195	197	QEL	196	196	E
199	200	CI	199	201	CIK
222	233	GPQITSPALNKL	222	233	GPQITSPALTQL
250	260	LTKLGVGNNQL	250	262	LTKLGVGNNQLSS
269	269	I	269	269	I
328	339	SVIEELDTSYCI	328	340	SVIEELDTSYCIE
348	357	TRIVTFPMSP	348	357	TRIVTFPMSP
364	364	S	NO	NO	NO
367	370	TSAC	367	370	TSAC
372	380	YSKTEGALT	372	380	YSKTEGALT
408	413	ISQNYG	407	413	IISQNYG
442	462	VTYQKNISIQDSQVIITGNLD	441	464	DATYQKNISILDSQVIITGNLDIS
482	483	EE	NO	NO	NO
486	490	RKLDK	489	496	DKVNVRLT
529	549	QKAQQKTLLWLGNNTLDMRA	529	550	QKAQQKTLLWLGNNTLDMRAT

NO: Not predicted

Table 2. The B cell epitope prediction of NDV hemagglutinin-neuraminidase

LaSota			Genotype VII		
Start	End	Peptide	Start	End	Peptide
7	18	QVALENDEREAK	5	18	VNRVVLENEEREAK
49	59	GASTPSDLVGI	53	58	PHDLAG
61	61	T	65	79	KTEDKVTSLSSSQD
67	69	EEK	82	82	D
75	76	GS	90	90	L
79	79	D	98	98	N
108	158	TLSYQINGAANNSGCGAPIHDPDY IGGIGKELIVDDASDVTSFYPSAFQG	111	140	SYQINGAANNSGCGAPVHDPDYIGGIGKEL
161	170	NFIPAPTTGS	142	170	VDNISDVTSFYPSAYQEHLNFIPAPTTGS
196	201	CRDHS	196	201	CRDHS
NO	NO	NO	216	217	TG
228	235	NLDDTQNR	228	235	NLDDTQNR
256	267	ETEEEDYNSAVP	256	267	GTEEDYKSVAP
281	291	YHEKDLDTVTL	281	292	YHEKDLDTVTLF
321	365	KPNSPSDTVQEGKYVIYKRYNDT CPDEQDYQIRMAKSSYKPRFG	321	365	KPNSPSDTAQEGKYVIYKRHNNA CPDQQDYQIRMAKSSYKPRFG
380	385	TSLGED	380	385	TSLGKD
420	421	YF	NO	NO	NO
433	457	NKTATLHSPYTFNAFTRPGSIPCQA	433	458	NKTATLYSPYKFNAFTRPGSIPCQAS
460	460	R	460	460	R
478	483	FYRNHT	479	482	HRNH
495	498	VQAR	494	499	DEQARL
515	523	TRVSSSSTK	517	523	VSSSIK
552	554	LFG	553	554	FG
567	574	KDDGVREA	NO	NO	NO

NO: Not predicted

Table 3. The MHC-I binding predictions of NDV fusion protein to the most frequent HLA-A and –B alleles.

LaSota				Genotype VII			
Start	Peptide	Allele	IC ₅₀	Start	Peptide	Allele	IC ₅₀
9	NPAPMTLTI	HLA-A*53:01	31.31	6	STRIPVPLR	HLA-A*31:01 HLA-A*30:01	10.26 25.59
14	TLTIRVALV	HLA-A*02:03	27.62	14	RLITRVMLI	HLA-A*02:03 HLA-A*32:01 HLA-A*02:01	8.32 17.03 30.39
21	LVLSCICPA	HLA-A*02:06	16.58	18	RVMLILSCI	HLA-A*32:01 HLA-A*02:06	32.40 34.31
35	RPLAAAGIV	HLA-B*07:02	31.13	35	RPLAAAGIV	HLA-B*07:02	31.13
53	YTSSQTGSI	HLA-A*68:02	41.71	53	YTSSQTGSI	HLA-A*68:02	39.59
73	KEACAKAPL	HLA-B*40:01	14.26	73	KEACAKAPL	HLA-B*40:01	14.26
87	TLTLLTPL	HLA-A*02:03 HLA-A*02:01	12.93 32.48	87	TLTLLTPL	HLA-A*02:03 HLA-A*02:01	12.93 31.97
117	LIGAIIGGV	HLA-A*02:03	13.59	117	FIGAVIGSV	HLA-A*02:03 HLA-A*02:06 HLA-A*02:01	4.13 14.03 22.94
124	SIAATNEAV	HLA-A*68:02 HLA-A*02:03	44.29 48.73	124	SIAATNEAV	HLA-A*68:02 HLA-A*02:03	43.01 48.06
131	TAAQITAAA	HLA-A*68:02	19.83	131	TAAQITAAA	HLA-A*68:02	19.83
133	AQITAAAAL	HLA-A*02:06 HLA-B*15:01	24.19 24.85	133	AQITAAAAL	HLA-A*02:06 HLA-B*15:01	24.19 24.85
137	AAAALIQAK	HLA-A*11:01	28.68	NO	NO	NO	NO
145	KQNAANILR	HLA-A*31:01	17.76	147	NAANILRLK	HLA-A*68:01	10.24
153	RLKESIAAT	HLA-A*02:03	17.57	153	RLKESIAAT	HLA-A*02:03	17.57
156	ESIAATNEA	HLA-A*68:02	25.32	156	ESIAATNEA	HLA-A*68:02	25.32
157	ASALITYIV	HLA-A*68:02 HLA-A*02:03	44.29 48.73	157	ASALITYIV	HLA-A*68:02	44.29
160	ATNEAVHEV	HLA-A*02:06 HLA-A*02:03 HLA-A*68:02	14.16 22.63 30.93	160	ATNEAVHEV	HLA-A*02:06 HLA-A*02:03 HLA-A*68:02	14.16 22.63 30.93
171	GLSQLAVAV	HLA-A*02:03 HLA-A*02:01	12.32 36.41	171	GLSQLAVAV	HLA-A*02:03 HLA-A*11:01 HLA-A*02:01	12.32 15.41 35.58
173	SQLAVAVGK	HLA-A*11:01	24.25	173	SQLAVAVGK	HLA-A*11:01	24.25
182	MQQFVNDQF	HLA-B*15:01	41.85	182	MQQFVNDQF	HLA-B*15:01	40.33
213	YLTELTTVF	HLA-B*15:01 HLA-B*35:01	20.50 48.73	213	YLTELTTVF	HLA-B*15:01 HLA-B*35:01	20.50 48.73
222	GPQITSPAL	HLA-B*07:02	17.99	222	GPQITSPAL	HLA-B*07:02	17.99
227	SPALNKLTI	HLA-B*07:02	31.02	227	TQLTIQALY	HLA-A*30:02	31.97
233	LTIQALYNL	HLA-A*02:06	25.37	233	LTIQALYNL	HLA-A*02:06	25.37
274	ILYDSQTQL	HLA-A*02:03 HLA-A*02:01	10.16 15.06	274	ILYDSQTQL	HLA-A*02:03 HLA-A*02:01	10.16 15.06
281	QLLGIQVTL	HLA-A*02:01	31.08	281	QLLGIQVTL	HLA-A*02:01	31.08
284	GIQVTLPSV	HLA-A*02:06 HLA-A*02:03	38.85 42.81	284	GIQVTLPSV	HLA-A*02:06 HLA-A*02:03 HLA-A*02:03	36.68 41.85 42.81
291	SVGNLNNMR	HLA-A*68:01	36.68	291	SVGNLNNMR	HLA-A*68:01	36.40
300	ATYLETLSV	HLA-A*02:06	48.06	NO	NO	NO	NO
304	ETLSVSTTK	HLA-A*68:01 HLA-A*33:01	7.80 29.04	304	ETLSVSTTK	HLA-A*68:01	7.80
314	FASALVPKV	HLA-A*02:06 HLA-A*02:03 HLA-A*68:02	11.70 29.34 40.33	314	FASALVPKV	HLA-A*02:06 HLA-A*02:03 HLA-A*68:02	11.70 29.34 38.85
321	KVVTQVGSV	HLA-A*02:03	35.05	321	KVVTQVGSV	HLA-A*02:03	33.09
345	LYCTRIVTF	HLA-A*23:01 HLA-A*24:02	33.09 42.15	345	LYCTRIVTF	HLA-A*23:01 HLA-A*24:02	32.48 41.71
347	EVTDGLSQL	HLA-A*68:02	46.52	347	EVTDGLSQL	HLA-A*68:02	46.48
366	NTSACMYSK	HLA-A*68:01 HLA-A*11:01	7.59 19.87	366	NTSACMYSK	HLA-A*68:01 HLA-A*11:01	7.59 19.87
370	CMYSKTEGA	HLA-A*02:03	11.94	370	CMYSKTEGA	HLA-A*02:03	11.94
379	LTPPYMALK	HLA-A*11:01	46.45	379	LTPPYMALK	HLA-A*68:01	12.17

LaSota				Genotype VII			
Start	Peptide	Allele	IC ₅₀	Start	Peptide	Allele	IC ₅₀
384	LTPPYMALK	HLA-A*68:02	22.29	383	YMALKGSVI	HLA-A*02:03 HLA-B*08:01	18.37 38.30
421	MTIKGSVIA	HLA-A*02:06	35.50	427	LSIDGITLR	HLA-A*68:01	13.47
NO	NO	NO	NO	442	ATYQKNISI	HLA-A*32:01	35.05
472	KQSCNVLSL	HLA-A*11:01	40.11	480	KLAESNSKL	HLA-A*02:03	12.99
487	NSISNALNK	HLA-A*02:01	47.10	487	KLDKVNVR	HLA-A*02:01	36.41
NO	NO	NO	NO	490	KVNVRLTSA	HLA-A*30:01	23.22
494	KLDKVNVR	HLA-A*02:03	27.46	494	RLTSASALI	HLA-A*02:03	31.32
497	KLTSTSALI	HLA-A*68:02	7.18	497	SASALITYI	HLA-A*68:02	31.32
		HLA-A*02:03	39.18				
		HLA-A*68:01	49.11				
498	SASALITYI	HLA-A*30:01	46.48	498	CTRIVTFPM	HLA-A*30:01	44.88
500	CTRIVTFPM	HLA-A*02:03	47.01	500	ALITYIVLT	HLA-A*02:03	46.64
		HLA-A*02:01	49.62			HLA-A*02:01	48.77
NO	NO	NO	NO	501	LITYIVLTV	HLA-A*02:03	28.08
505	ALITYIVLT	HLA-A*02:06	15.78	505	IVLTVISLV	HLA-A*02:06	16.30
		HLA-A*02:03	33.52			HLA-A*02:03	33.03
		HLA-A*02:01	42.93			HLA-A*02:01	46.52
508	IVLTVISLV	HLA-A*68:02	8.01	508	TVISLVFGA	HLA-A*68:02	8.02
		HLA-A*02:06	9.93			HLA-A*02:06	15.81
		HLA-A*02:01	22.40				
		HLA-A*02:03	29.89				
512	TVISLVFGA	HLA-A*68:02	17.94	512	LVFGALS LV	HLA-A*02:03	5.67
		HLA-A*02:03	23.35			HLA-A*02:06	5.72
		HLA-A*02:01	27.04			HLA-A*68:02	10.15
						HLA-A*02:01	22.47
520	LVFGALS LI	HLA-A*03:01	8.35	520	VLACYL MYK	HLA-A*03:01	8.76
		HLA-A*11:01	14.96			HLA-A*11:01	12.93
530	VLACYL MYK	HLA-B*58:01	5.91	530	KAQQKTLLW	HLA-B*58:01	5.91
		HLA-B*57:01	18.02			HLA-B*57:01	18.02
536	KAQQKTLLW	HLA-A*02:01	27.95	536	LLWLGNTL	HLA-A*02:01	27.95

NO: Not predicted

The cutoff for the peptide selection was < 50.

Table 4. The MHC-II binding predictions of NDV fusion protein based on 7-allele method.

LaSota			Genotype VII		
Start	Peptide	Median consensus percentile	Start	Peptide	Median consensus percentile
11	APMTLTIRVALVLSC	15.0	11	VPLRLITRVMILILSC	9.8
61	IIVKLLPNMPKDKEA	11.0	61	IIVKLLPNMPKDKEA	9.8
NO	NO	NO	96	GDSIRRIQGSVSTSG	15.0
NO	NO	NO	111	GRRRKRFIGAVIGSV	18.0
NO	NO	NO	116	RFIGAVIGSVALGVA	17.0
311	TKGFASALVPKVVVTQ	19.0	311	TKGFASALVPKVVVTQ	19.0
381	TPYMALKGSVIANCK	16.0	381	TPYMALKGSVIANCK	12.0
NO	NO	NO	491	VNVRLTSASALITYI	14.0
501	LITYIVLTVISLVFG	15.0	501	LITYIVLTVISLVFG	17.0
511	SLVFGILSLILACYL	17.0	NO	NO	NO

NO: Not predicted

The cutoff for the peptide selection was < 20.

Similar predictions were applied for HN protein (Tables 5 and 6). The top epitopes in both genotypes that linked with more alleles were located in the middle to the end of the protein sequence. Only two epitopes residues of HN protein from

MHC-I at positions 491 and 501 were overlapped with MHC-II epitopes in genotype VII, while the number of overlapping was increased to eight for the LaSota strain.

Table 5. The MHC-I binding predictions of NDV hemagglutinin-neuraminidase protein to the most frequent HLA-A and -B alleles. The cutoff for the peptide selection was ≤ 50 .

LaSota				Genotype VII			
Start	Peptide	Allele	IC ₅₀	Start	Peptide	Allele	IC ₅₀
15	REAKNT WRL	HLA-B*40:01	18.33	15	REAKNTWR L	HLA-B*40:01	18.33
18	KNTWRL IFR	HLA-A*31:01	17.53	18	KNTWRLVF R	HLA-A*31:01	14.79
		HLA-A*68:02	34.04			HLA-A*68:02	32.97
22	RLIFRIAI L	HLA-A*02:03	26.11	22	RLVFRITVL	HLA-A*02:03	39.28
		HLA-A*02:06	31.32			HLA-A*68:02	48.71
		HLA-A*02:03	37.65				
31	FLTVVT LAI	HLA-A*02:03	12.78	31	LLMVMTLA I	HLA-A*02:01	18.63
		HLA-A*02:01	14.51			HLA-A*02:03	24.11
		HLA-A*02:06	47.99			HLA-A*68:02	25.11
		HLA-A*68:02	17.05			HLA-A*02:06	35.36
36	TLAISVA SL	HLA-A*02:03	17.31	35	MTLAISAA A	HLA-A*68:02	5.69
						HLA-A*02:06	27.10
38	AISVASL LY	HLA-A*11:01	46.87	38	AISAAALA Y	HLA-B*15:01	47.43
		HLA-A*30:02	48.25				
40	SVASLL YSM	HLA-A*02:06	24.78	40	SAAALAYS M	HLA-B*35:01	14.17
		HLA-A*68:02	25.50				
		HLA-A*02:03	44.46				
66	AEEKITS TL	HLA-B*40:01	13.98	66	TEDKVTSL L	HLA-B*40:01	24.18
NO	NO	NO	NO	72	SLLSSSQDV	HLA-A*02:03	38.51
99	TETTIM NAI	HLA-B*40:01	40.27	99	TESIIMNAI	HLA-B*40:01	38.29
102	TIMNAIT SL	HLA-A*02:03	19.79	102	IIMNAITSL	HLA-A*02:03	12.47
		HLA-A*02:06	27.78			HLA-A*02:06	16.05
						HLA-A*02:01	27.14
109	SLSYQIN GA	HLA-A*02:03	30.01	109	SLSYQINGA	HLA-A*02:03	31.01
NO	NO	NO	NO	140	LIVDNISDV	HLA-A*02:03	31.01
144	DASDVT SFY	HLA-B*35:01	22.96	144	NISDVTSFY	HLA-A*68:01	38.08
		HLA-A*26:01	47.80			HLA-A*26:01	45.33
NO	NO	NO	NO	148	VTSFYPSAY	HLA-A*30:02	24.15
						HLA-A*01:01	42.08

LaSota				Genotype VII			
Start	Peptide	Allele	IC ₅₀	Start	Peptide	Allele	IC ₅₀
154	SAFQGHLENF	HLA-B*35:01	27.98	152	YPSAYQEHL	HLA-A*24:02	33.17
159	HLNFIAPPT	HLA-B*15:01	47.96	159	HLNFIAPPT	HLA-B*35:01	49.69
NO	NO	NO	NO	199	HSLSHQYLA	HLA-A*02:03	47.96
203	YQYLALGV L	HLA-A*02:06	33.83	203	HQYLALGVL	HLA-A*30:01	23.51
		HLA-A*31:01	35.72			HLA-A*31:01	35.72
		HLA-A*33:01	38.71			HLA-A*33:01	38.71
		HLA-A*02:03	39.11			HLA-A*02:03	39.11
261	DYNSAVPT R	HLA-A*33:01	32.97	NO	NO	NO	NO
269	RMVHGRLG F	HLA-B*15:01	13.10	269	SMVHGRLGF	HLA-B*15:01	21.70
		HLA-A*32:01	21.76	288	TTVLFKDWV	HLA-A*68:02	21.93
NO	NO	NO	NO	299	YPGVGGGSF	HLA-B*35:01	19.38
299	YPGVGGGS F	HLA-B*35:01	19.38	299	YPGVGGGSF	HLA-B*07:02	33.86
		HLA-B*07:02	33.86			305	GSFIDSRVW
305	GSFIDSRVW	HLA-B*58:01	11.48	305	GSFIDSRVW	HLA-B*57:01	24.60
		HLA-B*57:01	19.43			354	MAKSSYKPK
NO	NO	NO	NO	368	RVQQAILSI	HLA-A*32:01	17.30
368	RIQQAILSI	HLA-A*32:01	20.31	404	ILTVGTSHF	HLA-A*68:02	27.55
404	ILTVGTSHF	HLA-A*68:02	27.55			HLA-A*30:02	34.04
		HLA-A*30:02	34.04			HLA-B*15:01	42.82

408	GTSHFLYQR	HLA-A*31:01	8.76	408	GTSHFLYQR	HLA-A*31:01	8.76
		HLA-A*68:01	15.89			HLA-A*68:01	15.89
		HLA-A*11:01	21.55			HLA-A*11:01	21.55
412	FLYQRGSSY	HLA-B*15:01	7.78	412	FLYQRGSSY	HLA-B*15:01	7.78
		HLA-A*24:02	14.61			HLA-A*24:02	14.61
		HLA-A*23:01	21.33			HLA-A*23:01	21.33
		HLA-B*35:01	43.63			HLA-B*35:01	43.63
419	SYFSPALLY	HLA-A*30:02	32.22	419	SYFSPALLY	HLA-A*30:02	32.22
434	KTATLHSPY	HLA-A*30:02	9.25	434	KTATLHSPY	HLA-A*30:02	9.25
		HLA-A*32:01	18.14			HLA-A*32:01	18.14
		HLA-B*15:01	25.84			HLA-B*15:01	25.84
		HLA-B*58:01	31.24			HLA-B*58:01	31.24

LaSota				Genotype VII			
Start	Peptide	Allele	IC ₅₀	Start	Peptide	Allele	IC ₅₀
469	VYTDPYPLI	HLA-A*01:01	12.22	469	VYTDPYPLI	HLA-A*01:01	12.22
		HLA-A*24:02	35.66			HLA-A*24:02	35.66
		HLA-A*23:01	39.53			HLA-A*23:01	39.53
476	LIFYRNHTL	HLA-A*31:01	11.63	476	LIFYRNHTL	HLA-A*33:01	21.35
		HLA-A*33:01	21.35			HLA-B*08:01	23.80
		HLA-B*08:01	23.80				
491	MLDGVQARL	HLA-A*02:01	20.09	491	MLDGVQARL	HLA-A*02:01	29.10
498	RLNPASAVF	HLA-B*15:01	5.01	498	RLNPASAVF	HLA-B*15:01	5.01
		HLA-A*32:01	13.71			HLA-A*32:01	7.79
503	SAVFDSTSR	HLA-A*68:01	18.50	503	SAVFDSTSR	HLA-A*68:01	14.69
508	STSRSRITR	HLA-A*30:01	7.13	508	STSRSRITR	HLA-A*31:01	18.22
		HLA-A*31:01	15.99			HLA-A*68:01	30.49
		HLA-A*68:01	16.68			HLA-A*31:01	46.55
NO	NO	NO	NO	511	RSRVTRVSS	HLA-A*30:01	7.38
518	SSSSTKAAY	HLA-A*30:01	23.35	518	SSSIKAAY	HLA-A*30:02	29.48
524	AAYTTSTCF	HLA-A*68:02	12.41	524	AAYTTSTCF	HLA-A*68:02	12.41
		HLA-A*02:06	20.40			HLA-A*02:06	20.40
		HLA-B*15:01	46.76			HLA-B*15:01	46.76
536	KTNKTYCLS	HLA-A*30:01	43.94	536	KTNKTYCLS	HLA-A*30:01	43.86
544	SIAEISNTL	HLA-A*02:03	18.61	544	SIAEISNTL	HLA-A*02:03	18.61
		HLA-A*68:02	27.37			HLA-A*68:02	27.37
		HLA-A*02:06	42.95			HLA-A*02:06	42.95
551	TLFGFRIV	HLA-A*02:03	10.07	551	TLFGFRIV	HLA-A*02:03	10.07
		HLA-B*40:01	12.29			HLA-B*40:01	12.29
		HLA-A*02:01	47.49			HLA-A*02:01	47.49
557	RIVPLLVEI	HLA-A*02:06	14.23	NO	NO	NO	NO
		HLA-A*32:01	29.71				
		HLA-A*02:03	35.81				

NO: Not predicted

The cutoff for the peptide selection was <

Table 6. The MHC-II binding predictions of NDV hemagglutinin-neuraminidase protein based on 7-allele method.

LaSota			Genotype VII		
Start	Peptide	Median consensus percentile	Start	Peptide	Median consensus percentile
16	EAKNTWRLIFRIAIL	17.0	NO	NO	NO
21	WRLIFRIAILFLTVV	7.6	21	WRLVFRITVLLLMVM	14.0
26	RIAILFLTVVTLAIS	18.0	NO	NO	NO
31	FLTVVTLAISVASLL	16.0	31	LLMVMTLAISAAAALA	6.7
NO	NO	NO	91	ESPLALLNTESIIMN	18.0
NO	NO	NO	96	LLNTESIIMNAITSL	17.0
101	TTIMNAITSLSYQIN	11.0	101	SIIMNAITSLSYQIN	9.8
NO	NO	NO	136	IGKELIVDNISDVTS	11.0
206	LALGVLRTSATGRVF	7.3	206	LALGVLRTSATGRVF	7.3
216	TGRVFFSTLRSINLD	14.0	216	TGRVFFSTLRSINLD	14.0
221	FSTLRSINLDDTQNR	18.0	221	FSTLRSINLDDTQNR	18.0
NO	NO	NO	291	LFKDWVANYPGVGGG	19.0
346	DEQDYQIRMAKSSYK	19.0	346	DQDYQIRMAKSSYK	17.0
NO	NO	NO	351	QIRMAKSSYKPKRFG	20.0
366	GKRIQQAILSIVST	16.0	366	GKRVQQAILSIVST	17.0
371	QAILSIVSTSLGED	17.0	371	QAILSIVSTSLGKD	12.0
476	LIFYRNHTLRGVFGT	15.0	501	PVSAVFDSISRRT	18.0
531	CFKVVKTNKTYCLSI	13.0	526	YTTSTCFKVVKTNKV	17.0
551	TLFGEFRIVPLLVEI	18.0	545	CFKVVKTNKVYCLSI	8.0

NO: Not predicted

The cutoff for the peptide selection was <20.

The Secondary Structure Predictions

The predicted secondary structures of F and HN proteins in alpha helix, extended strand, and random coil features were relatively similar in both genotypes (Fig. 1). The transmembrane topology analysis of F protein revealed that the number of transmembrane helices was varied between the two

genotypes. As shown in Fig. 2, one helix with the lengths of amino acid sequences at 117–139 was predicted for genotype II. In addition to this sequence, another helix at 503–525 was also predicted for genotype VII. The HN protein had one transmembrane helix at 23–45 in both genotypes.

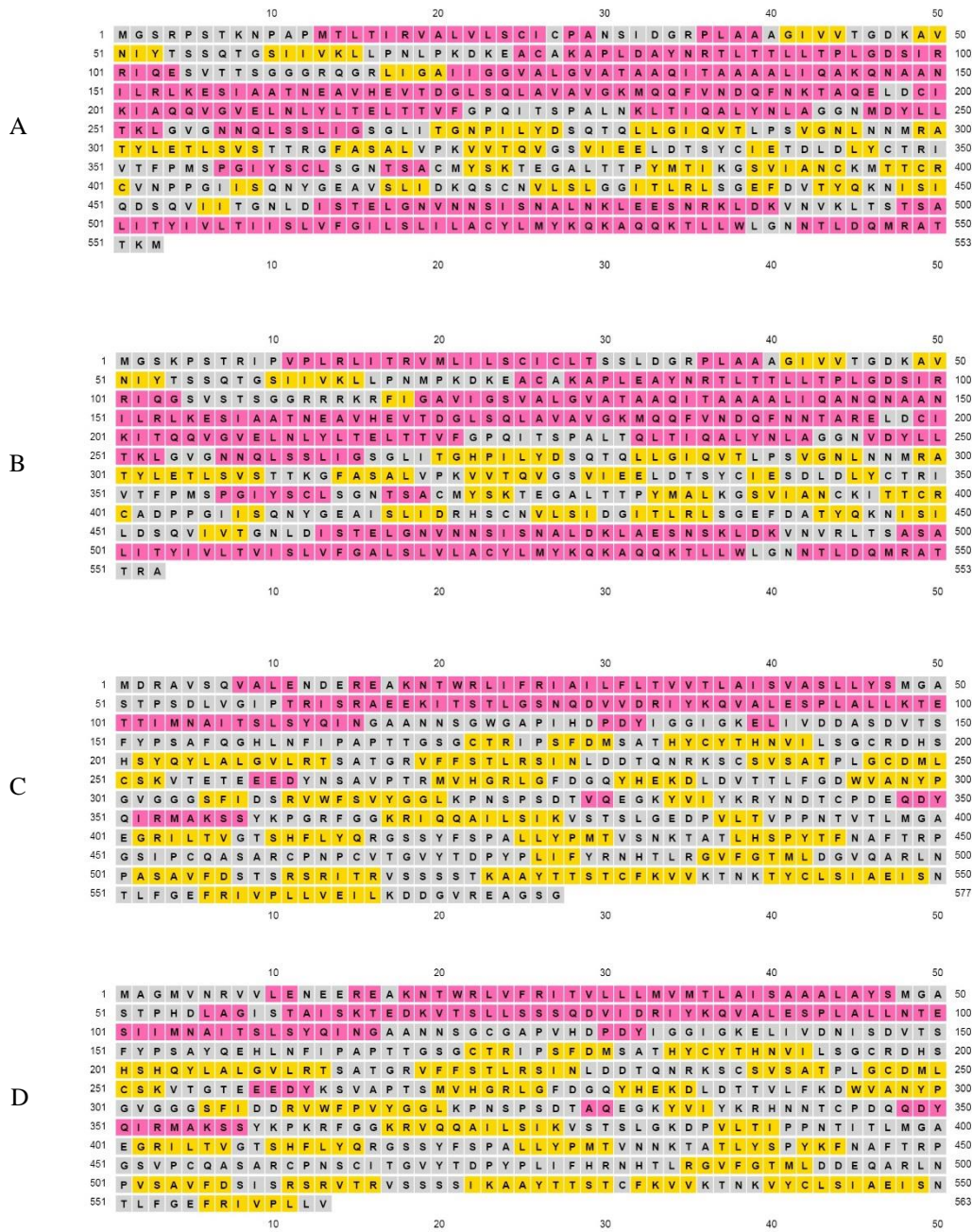


Fig. 1. Secondary structures of NDV fusion protein of genotypes II and VII (A, B) and hemagglutinin-neuraminidase protein of genotypes II and VII (C, D). The helix, strand, and coil are shown in pink, yellow, and gray, respectively.

[Downloaded from vacres.pasteur.ac.ir on 2024-04-23]

[DOI: 10.52547/vacres.8.2.4]

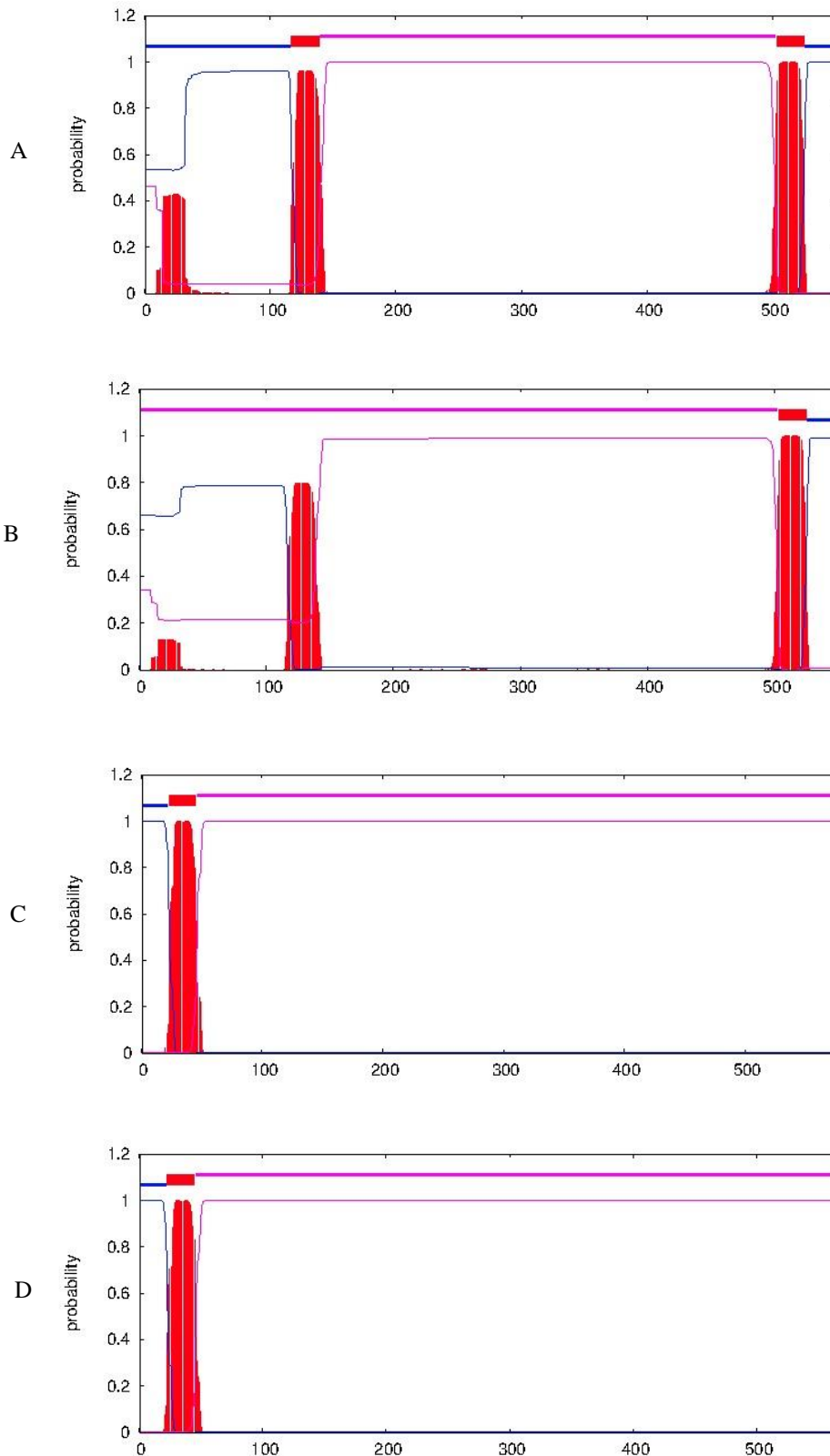


Fig. 2. Transmembrane helices of NDV fusion protein of genotypes II and VII (A, B) and hemagglutinin-neuraminidase protein of genotypes II and VII (C, D). The transmembrane, inside, and outside are shown with red, blue, and pink lines, respectively.

According to the results of physicochemical parameters analysis shown in Table 7, the theoretical pI, and the negatively and positively charged of each F and HN proteins were similar between the NDV genotypes. The instability index of these proteins was ranged between 30.69 and 37.64. A level smaller than 40 indicates the potential stability of these proteins, which

is confirmed by the positive aliphatic index. Relative volumes occupied by aliphatic side chain amino acids (alanine, isoleucine, leucine, proline, and valine) of F and HN proteins were positive for both genotypes. GRAVY index indicates the solubility of the proteins and is calculated as the sum of the hydrophathy values for all the amino acids in a protein divided

by the total number of residues in it. The predicted GRAVY values indicate that the HN protein is non-polar and hydrophilic, and the F protein is polar and hydrophobic.

Moreover, the LaSota HN protein had a moderate aliphatic index (~80) which indicates stability in a wide spectrum of temperatures.

Table 7. Physicochemical parameters analysis of NDV fusion protein and hemagglutinin-neuraminidase protein of genotypes II and VII.

	Theoretical isoelectric point	Negatively charge	Positively charge	Instability index	Aliphatic index	GRAVY
F II	8.53	37	43	37.67	109.17	0.188
F VII	8.58	38	44	33.78	110.76	0.223
HN II	7.53	52	53	30.69	81.09	-0.152
HN VII	8.64	46	53	34.00	83.11	-0.125

DISCUSSION

Prevention of avian respiratory viral diseases such as ND is important for public health worldwide. Genetic divergence and the antigenic difference between the field isolates and vaccine strain arise the question of insufficient protection against NDV in areas of intense poultry production. Characterization of the molecular properties of NDV isolates along with analyzing the virus proteins in affinity binding to B- and T-cells are crucial for proposing appropriate approaches for designing of an effective vaccine. The difference in antigenic determinants to trigger immune responses and production of protective antibodies is the main factor that defines the poor cross-immunity between the vaccine and field virus strains [22-24]. The F and HN of NDV are the major immunogens and responsible for the induction of virus-neutralizing antibody responses [25, 26]. In this study, we used bioinformatics analyses to evaluate the possible impacts of genetic divergence between the genotype VII and LaSota vaccine strain on the immunity against NDV by focusing on the F and HN glycoproteins.

Results associated with B-cell epitopes showed that there were many similarities between the predicted antigenic epitopes in the F protein of these genotypes. However, the antigenicity value was higher for the HN LaSota strain than genotype VII virus. The B-cell epitopes with high antigenicity values were located at position 108 to the end of the LaSota HN sequence, which possessed the ability to bind MHC-I and/or MHC-II. Such associations with both B- and T-cells indicate how a traditional live attenuated NDV vaccine (prepared by genotype II) can induce neutralizing antibody responses and also provide protection against NDV.

Protein structure plays a significant role in the critical functions of viruses and any structural changes may directly influence them. The genomic diversity between genotype II and genotype VII did not alter secondary structures of F and HN proteins. In search for transmembrane helix within the sequences, a helical motif in the range of 17–25 amino acids was proposed at positions 23–45 for HN protein for the both genotypes. Interestingly, two helical domains were predicted for the F protein of genotype VII, one after the proteolytic cleavage site and another at the end of the sequence. The latter was not predicted for the LaSota strain. The addition of another helix is probably related to the differences in epitope prediction

or allelic binding at positions 421 to 512 between the two genotypes.

The transmembrane helices require multiple hydrophobic residues for peptide binding to MHC-I and the presence of these residues in close proximity of each other is a hallmark of the helices [27]. The predicted epitopes for genotype VII are predominantly hydrophobic, which may be the reason for the formation of another helix in this region. This finding could be responsible for any differences in immunological responses against two genotypes in fields. In physicochemical analysis, HN protein had a negative GRAVY value compared to the F protein indicating that HN is hydrophilic and accessible on the surface. The pI of proteins is mainly dependent on the polar amino acids in their structure. A protein with a pI of 7 will be positively charged and is accessible to surrounding water molecules, as predicted for the LaSota strain. Surface accessibility and characteristic solubility are important parameters for potential immunogenicity of a protein [28]. Compared to genotype VII, the lower GRAVY and pI values of HN in LaSota strain improve the solubility of the protein, resulting in better interaction with antibody molecules through polar interactions. Taken together, there was no major difference between the predicted antigenicity values, epitope regions, affinity binding to MHC-I and MHC-II, secondary structures, and physicochemical properties for these immunogens between the LaSota strain and genotype VII.

Poor cross-immunity between the vaccine virus and the field isolates does not seem the right claim in response to outbreaks of virulent NDV among vaccinated chickens. However, revising the NDV vaccination strategy is needed to increase the protection, reduce the viral shedding, and deal with the repeated outbreaks. Studies have been conducted to introduce the live genotype-matched vaccines that could be sufficient to protect against the prevalent and the newly emerging NDVs. This process is currently not realized because a genotype VII strain that is adapted to the embryonated egg propagation and gives optimal protection has not yet been introduced as an alternative to the classical strain. Since only the lentogenic NDV strains with an ICPI value greater than 0.4 are recommended as a vaccine strain [29], new vaccines have been developed through vector-based recombinant and reverse genetics techniques. These vaccine candidates include a recombinant plant, fowlpox virus- and herpesvirus-vectored, and reverse genetic LaSota vaccines, expressing virulent F and/or HN proteins [14, 30, 15, 31, 16, 17]. Although the vaccines are able to protect chickens from virulent NDV

challenges and significantly reduce virus shedding in the vaccine efficacy trials, their administrations are affected by the cell-associated factors, and the presence of maternal antibodies, which neutralize the antigenicity of the vaccine [32]. Intensive immunizations with the commercially NDV vaccines have revealed that the genotype II viruses can still provide a good protection level against genotype VII viruses; hence, the use of conventional vaccines in ND control programs is inevitable. Due to the importance of local mucosal immunity induced by live vaccines, a combination schedule of attenuated vaccine and the inactivated vaccine has been suggested to gain better protection against virulent NDVs [32, 33].

Current NDV vaccines are consisted of live attenuated and inactivated genotype II and genotype I-based viruses, which are used depending on the disease situation and the national requirements. It has been suggested that the genotype VII-matched vaccines provide better protection from NDV challenge with the highest cell-mediated immune response and HI titers [34, 35, 24]. In conclusion, the use of genotype VII inactivated vaccine instead of genotype I might help to control frequent ND outbreaks. However, to screen a suitable vaccine seed candidate, many passages in the chicken embryo or cell line should be applied. Besides the removal of limiting factors in the development of a genotype-matched vaccine, proper compliance biosecurity practices are also necessary to prevent the failure of a vaccination strategy.

ACKNOWLEDGEMENT

This work was supported by Razi Vaccine and Serum Research Institute under grant No.01-18-18-035-95-17.

CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

REFERENCES

- Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetics and Evolution*. 2019;74:103917. doi: 10.1016/j.meegid.2019.103917.
- Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A et al. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infection, Genetics and Evolution*. 2015;29:216-29. doi: 10.1016/j.meegid.2014.10.032.
- Miller PJ, Kim LM, Ip HS, Afonso CL. Evolutionary dynamics of Newcastle disease virus. *Virology*. 2009;391(1):64-72. doi: 10.1016/j.virol.2009.05.033.
- Steward M, Vipond IB, Millar NS, Emmerson PT. RNA editing in Newcastle disease virus. *Journal of General Virology*. 1993;74(12):2539-47. doi: 10.1099/0022-1317-74-12-2539.
- Khattar SK, Yan Y, Panda A, Collins PL, Samal SK. A Y526Q mutation in the Newcastle disease virus HN protein reduces its functional activities and attenuates virus replication and pathogenicity. *Journal of Virology*. 2009;83(15):7779-82. doi: 10.1128/JVI.00536-09.
- Panda A, Huang Z, Elankumaran S, Rockemann DD, Samal SK. Role of fusion protein cleavage site in the virulence of Newcastle disease virus. *Microbial Pathogenesis*. 2004;36(1):1-10. doi: 10.1016/j.micpath.2003.07.003.
- Heiden S, Grund C, Röder A, Granzow H, Kühnel D, Mettenleiter TC et al. Different regions of the newcastle disease virus fusion protein modulate pathogenicity. *PLoS One*. 2014;9(12):e113344. doi: 10.1371/journal.pone.0113344.
- Toyoda T, Sakaguchi T, Imai K, Inocencio NM, Gotoh B, Hamaguchi M et al. Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus. *Virology*. 1987;158(1):242-7. doi: 10.1016/0042-6822(87)90261-3.
- Al-Garib S, Gielkens A, Gruys E, Kochi GJ. Review of Newcastle disease virus with particular references to immunity and vaccination. *World's Poultry Science Journal*. 2003;59(2):185-200. doi: 10.1079/WPS20030011.
- Ebrahimi MM, Shahsavandi S, Moazenijula G, Shamsara M. Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008–2011. *Virus Genes*. 2012;45(1):63-8. doi: 10.1007/s11262-012-0738-5.
- Herczeg J, Wehmann E, Bragg R, Travassos Dias P, Hadjiev G, Werner O et al. Two novel genetic groups (VIIb and VIII) responsible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe. *Archives of Virology*. 1999;144(11):2087-99. doi: 10.1007/s007050050624.
- Steenfels M, Van Borm S, Mertens I, Houdart P, Rauw F, Roupie V et al. Molecular and virological characterization of the first poultry outbreaks of Genotype VII. 2 velogenic avian orthoavulavirus type 1 (NDV) in North West Europe, BeNeLux, 2018. *Transbound and Emerging Diseases*. 2021;68(4):2147-60. doi:10.1111/tbed.13863.
- Xue C, Cong Y, Yin R, Sun Y, Ding C, Yu S et al. Genetic diversity of the genotype VII Newcastle disease virus: identification of a novel VIIj sub-genotype. *Virus Genes*. 2017;53(1):63-70. doi: 10.1007/s11262-016-1404-0.
- Bello MB, Yusoff K, Ideris A, Hair-Bejo M, Peeters BP, Omar AR. Diagnostic and vaccination approaches for Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed Research International*. 2018;2018:7278459. doi: 10.1155/2018/7278459.
- Huang Z, Elankumaran S, Panda A, Samal SJ. Recombinant Newcastle disease virus as a vaccine vector. *Poultry Science*. 2003;82(6):899-906. doi: 10.1093/ps/82.6.899.
- Motamedi MJ, Ebrahimi MM, Shahsavandi S, Amani J, Kazemi R, Jafari M et al. The immunogenicity of a novel chimeric hemagglutinin-neuraminidase-fusion antigen from Newcastle disease virus by oral delivery of transgenic canola seeds to chickens. *Molecular Biotechnology*. 2020;62(6):344-54. doi: 10.1007/s12033-020-00254-y.
- Wang J, Wang C, Feng N, Wang H, Zheng X, Yang S et al. Development of a reverse genetics system based on RNA polymerase II for Newcastle disease virus genotype VII. *Virus Genes*. 2015;50(1):152-5. doi: 10.1007/s11262-014-1137-x.
- Kim Y, Ponomarenko J, Zhu Z, Tamang D, Wang P, Greenbaum J et al. Immune epitope database analysis resource. *Nucleic Acids Research*. 2012;40(W1):W525-W30. doi: 10.1093/nar/gks438.
- McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. *Bioinformatics*. 2000;16(4):404-5. doi: 10.1093/bioinformatics/16.4.404.
- Käll L, Krogh A, Sonnhammer EL. Advantages of combined transmembrane topology and signal peptide prediction—the Phobius web server. *Nucleic Acids Research*. 2007;35(suppl_2):W429-W32. doi: 10.1093/nar/gkm256.
- Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch AJ. Protein identification and analysis tools on the ExPASy server. In: Walker, J.M., editor. *The Proteomics Protocols Handbook*, Humana Press. 2005., 571-607.
- Miller PJ, King DJ, Afonso CL, Suarez DL. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. *Vaccine*. 2007;25(41):7238-46. doi: 10.1016/j.vaccine.2007.07.017.
- Roohani K, Tan SW, Yeap SK, Ideris A, Bejo MH, Omar AR. Characterisation of genotype VII Newcastle disease virus (NDV) isolated from NDV vaccinated chickens, and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *Journal of Veterinary Science*. 2015;16(4):447-57. doi: 10.4142/jvs.2015.16.4.447.
- Sedeik M, Elbestawy A, El-Shall N, Abd El-Hack M, Saadeldin I, Swelum A. Comparative efficacy of commercial inactivated Newcastle disease virus vaccines against Newcastle disease virus genotype VII in broiler chickens. *Poultry Science*. 2019;98(5):2000-7. doi: 10.3382/ps/pey559.
- Miller PJ, Koch GJ. Newcastle disease. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V.L., editors. *Diseases of Poultry*. 13th edition. Ames, IA: Wiley-Blackwell in partnership with the American Association of Avian Pathologists. 2013; 89-138.
- Seal BS, King DJ, Meinersmann RJ. Molecular evolution of the Newcastle disease virus matrix protein gene and phylogenetic relationships among the paramyxoviridae. *Virus Research*. 2000;66(1):1-11. doi: 10.1016/s0168-1702(99)00119-7.

27. Bianchi F, Textor J, van den Bogaart GJ. Transmembrane helices are an overlooked source of Major Histocompatibility Complex Class I epitopes. *Frontiers in Immunology*. 2017;8:1118. doi: 10.3389/fimmu.2017.01118.
28. Naim JO, van Oss CJ. The effect of hydrophilicity-hydrophobicity and solubility on the immunogenicity of some natural and synthetic polymers. *Immunological Investigations*. 1992;21(7):649-62. doi: 10.3109/08820139209069401.
29. Council of the European Communities. Commission Decision of 8 February 1993 laying down the criteria to be used against Newcastle disease in the context of routine vaccination programmes. *The Official Journal of the European Union*. 1993;59:35.
30. Esaki M, Godoy A, Rosenberger JK, Rosenberger SC, Gardin Y, Yasuda A et al. Protection and antibody response caused by turkey herpesvirus vector Newcastle disease vaccine. *Avian Diseases*. 2013;57(4):750-5. doi: 10.1637/10540-032613-Reg.1.
31. Karaca K, Sharma JM, Winslow BJ, Junker DE, Reddy S, Cochran M et al. Recombinant fowlpox viruses coexpressing chicken type I IFN and Newcastle disease virus HN and F genes: influence of IFN on protective efficacy and humoral responses of chickens following in ovo or post-hatch administration of recombinant viruses. *Vaccine*. 1998;16(16):1496-503. doi: 10.1016/s0264-410x(97)00295-8.
32. Dimitrov KM, Afonso CL, Yu Q, Miller PJ. Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology*. 2017;206:126-36. doi: 10.1016/j.vetmic.2016.12.019.
33. Winterfield R, Dhillon A, Alby L. Vaccination of chickens against Newcastle disease with live and inactivated Newcastle disease virus. *Poultry Science*. 1980;59(2):240-6. doi: 10.3382/ps.0590240.
34. Fawzy M, Ali RR, Elfeil WK, Saleh AA, El-Tarabilli MMA. Efficacy of inactivated velogenic Newcastle disease virus genotype VII vaccine in broiler chickens. *Veterinary Research Forum*. 2020;11(2):113-120. doi: 10.30466/vrf.2019.95311.2295.
35. Hu Z, Hu S, Meng C, Wang X, Zhu J, Liu X. Generation of a genotype VII Newcastle disease virus vaccine candidate with high yield in embryonated chicken eggs. *Avian Diseases*. 2011;55(3):391-7. doi: 10.1637/9633-122410-Reg.1