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Novel Multi Epitopes Vaccine Candidates against Vesicular Stomatitis Virus through Reverse Vaccinology

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Abstract Vesicular stomatitis (VS) is a disease of horses, cattle and swine caused by vesicular stomatitis virus (VSV). The virus belongs to the genus *Vesiculovirus* of the family *Rhabdoviridae*. The disease has no treatment or vaccine. Therefore the aim of this study was to design multi-epitopes vaccine against vesicular stomatitis New Jersey virus using peptides of the glycoprotein to stimulate protective immune response. A total of 46 sequences of the Glycoprotein of VSV were retrieved from NCBI database. Sequences were aligned to determine the conservancy and to predict epitopes using IEDB analysis resource. Six epitopes were predicted as promising B cell epitopes since they fulfilled the criteria of surface accessibility, antigenicity and proposed as most probable B cell epitope. These epitopes were $_{393}$VLKTKQGYK$_{401}$, $_{147}$PHSVKVDEY$_{155}$, $_{454}$SKNPVEL$_{460}$, $_{240}$CRKPGYKL$_{247}$, $_{427}$HPHIE$_{431}$ and $_{505}$PIYKS$_{509}$. For T cell; four epitopes $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, $_{189}$GESVCSQLF$_{197}$ and $_{108}$CLEAIKAYK$_{116}$ were proposed as MHC-I epitopes since they interacted with the highest numbers of alleles and with high binding affinity. For MHC-II four epitopes namely $_{241}$LKNDLWFQI$_{255}$, $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, and $_{18}$IEIVFPQHT$_{26}$ were proposed as peptide vaccine since they interacted with high affinity to MHC-II alleles. It is noteworthy the epitopes $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$ were found interacting with both MHC-I and MHC-II. Thus they further used for docking with the equine haplotype molecules (ELA-A3) where they demonstrated lowest binding energy to the equine MHC class I molecule haplotype. To our knowledge there is no epitope based vaccine for the Vesicular stomatitis New Jersey Virus (VSV-NJ) via reverse vaccinology. In this study, twelve epitopes were proposed eliciting both humeral and cell mediated immunity and predicted to act as a promising peptide vaccine against VSV. Clinical trial is required to proof these epitopes as an efficient vaccine against vesicular stomatitis virus.

Keywords: *Vesicular stomatitis Virus (VSV), Epitope, Peptide vaccine, Immune epitope database (IEDB), NCBI*

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1. Introduction

Vesicular stomatitis (VS) is a disease of horses, cattle, and swine caused by vesicular stomatitis virus (VSV). The virus belongs to the genus *Vesiculovirus* of the family *Rhabdoviridae* [1,2]. The disease is transmitted by black flies (*Simulium* spp.), sand flies (*Lutzomyia spp.)* and biting midges (*Culicoides* spp.) [3,4]. Clinically the affected animals showed typical vesicular lesions on muzzle, tongue, lips, or coronary band and teats [5,6]. The VS is classified as A-list infectious disease of the Office International des Epizooties (OIE) with economically important contagious disease of livestock [7,8]. The disease is endemic in Central America and northern regions of South America. Recently outbreaks were reported between 2004- 2006 and the infected horses reached up to 78% and 68% in cattle [9,10].

Vesicular stomatitis virus (VSV) contains two main serotypes; New Jersey and Indiana vesicular stomatitis. The former serotype account for more than 80% of the clinical cases reported in endemic areas [11,12]. VSV is negative-sense single-stranded genome encodes five structural proteins, nucleoprotein (N), Phosphproteins (P), matrix protein (M), glycoprotein (G), and polymerase (L) [13]. Glycoprotein was used as a vaccine vector and it has been shown to be the main antigenic target for immune response to the virus [14-17].

Vaccination is usually considered to be the most effective method of preventing infectious diseases. The only vaccine available for the control of VS is an

inactivated preparation. This vaccine showed no successful results [18,19]. Efforts to create live attenuated vaccines, subunit- or DNA mediated vaccines against VSV was initiated but have limited success [20,21]. At present there is no satisfactory vaccine against VSV infection. Therefore successful approaches for vaccine design against vesicular stomatitis virus are highly required. Epitope-based vaccines can be constructed for B-cell to predict epitopes that mainly induce antibody production and the T-cell epitopes that induce cellular response and cytokine secretion as cytotoxic T-cells. The advantages of epitope-based vaccines; it focused on the immune response, enhancing immunity and avoiding undesirable epitopes and reducing costs. The peptide vaccines have been developed for many infectious diseases [22-31]. Therefore the aim of this study was to design a peptide vaccine against vesicular stomatitis New Jersey virus using peptides of its glycoprotein as an immunogen to stimulate protective immune response.

2. Materials and Methods

2.1. Retrieval of Protein Sequences

A total of 46 Glycoprotein sequences of New Jersey Vesicular stomatitis virus were retrieved from National Center for Biotechnology Information database (NCBI) (https://www.ncbi.nlm.nih.gov/protein/) in 19th September 2017. Most of these sequences were from USA. The retrieved sequences and their accession numbers as well as the date and the region of collection were listed in Table 1.

2.2. Phylogenetic Evolution

Phylogenetic tree of the retrieved sequences of the capsid proteins of HEV was created using MEGA7.0.26 (7170509) software [32]. The protein tree was constructed using maximum likelihood parameter in the software.

2.3. Determination of Conserved Regions

Multiple sequence alignment was used to obtain conserved regions in the retrieved sequences using Clustal-W as applied in the Bio-Edit program (version 7.2.5.0) [33]. Candidate epitopes were analyzed by different prediction tools at the Immune Epitope Database IEDB analysis resource (http://www.iedb.org/).

2.4. B-cell Epitope Prediction

B-cell epitopes were characterized by their antigenicity, hydrophilicity, surface accessibility and flexibility. Hence, the reference sequence of glycoprotein was analyzed by several B-cell prediction tools. BepiPred linear epitope prediction tools was used to predict linear epitopes [34,35], Emini surface accessibility prediction tool was used to predict surface epitopes [36], and kolaskar and tongaonker antigenicity tool was used to predict antigenic epitopes [37].

Table 1. Retrieved virus strains, their accession numbers and their area of collection

Accession number	Year	Country	Accession number	Year	Country
YP_009047084	1984	Honduras	AAG00863.1	1999*	USA
AFO67835.2	1984	Honduras	AAG00862.1	1999*	USA
AFO67850.1	1995	USA	AAG00861.1	1999*	USA
AFO67845.1	2005	USA	AAG00860.1	1999*	USA
AFO67840.1	1989	USA	AAG00859.1	1999*	USA
AFO67830	1992	Colombia	AAG00858.1	1999*	USA
AFO67825.1	2003	Costa Rica	AAG00857.1	1999*	USA
AFO67820.1	1992	Costa Rica	AAG00856.1	1999*	USA
AFO67815.1	1985	Panama	AAG00855.1	1999*	USA
AFO67810.1	1983	Nicaragua	AAG00854.1	1999*	USA
AMK37534.1	1998	Colombia	AAG00853.1	1999*	USA
AMK37529.1	2000	Colombia	AAG00852.1	1999*	USA
AMK37524.1	1998	Colombia	AAG00851.1	1999*	USA
AMK37519.1	1998	Colombia	AAG00850.1	1999*	USA
AMK37514.1	1998	Colombia	AAG00849.1	1999*	USA
AMK37509.1	1993	Colombia	AAG00848.1	1999*	USA
AMK37504.1	1985	Ecuador	AAG00847.1	1999*	USA
AKO63234.1	2014	USA	AAG00846.1	1999*	USA
AKA88567.1	2014	USA	AAG00845.1	1999*	USA
AIP90495.1	2014	USA	AAG00844.1	1999*	USA
AGS42188.1	2012	USA	AAG00843.1	1999*	USA
AAG00865.1	1999*	USA	ASR83121.1	2007	Mexico
AAG00864.1	1999*	USA	ASR83116.1	2008	Mexico

*Specific year of submission in the NCBI database.

2.5. T-Cell Epitope Prediction

T cell epitopes were predicted by tools available in Immune Epitope Database (IEDB) (tools.immuneepitope.org) which provides on Major Histocompatibility Complex (MHC) binding predictions. It is noteworthy in this study the human alleles in the IEDB were used to predict the MHC-1 and MHC-11 binding epitopes.

2.5.1. MHC Class I Binding Predictions

MHC I prediction tool has been used to analyze the preferential binding of Glycoprotein to MHC class I molecule. A different method for MHC-1 binding prediction was available in the Immune Epitope Database (IEDB). The artificial neural network (ANN) was used to calculate IC50 values of peptide binding to MHC-1 molecules and calculation of the HLA alleles [38,39]. Before prediction, all peptides length was set to 9 amino acids [30]. The alleles having binding affinity IC50 equal to or less than 300 nM were suggested as candidate epitopes.

2.5.2. MHC Class II Binding Predictions

Peptide binding analysis of MHC class II molecules was assessed by the IEDB MHCII prediction tool. Certain HLA-DR, HLA-DP, HLA-DQ alleles were analyzed [40,41]. MHCII binding prediction was achieved using NN-align method [42]. All epitopes that bind to many alleles at score equal to or less than 1000 half-maximal inhibitory concentration (IC50) were proposed as MHCII epitopes.

2.6. Homology Modeling

The reference sequence of Glycoprotein of VSV-NJ was used to create the 3D structure by Raptor X server at (http://raptorx.uchicago.edu/Structure Prediction/predict/) and UCSF Chimera (version 1.8) was used to visualize the selected epitopes belonging to the B- cell, MHC I, and MHC II [43].

2.7. Molecular Docking

Pep fold software was used to predict the three dimensional structure of selected peptides [44]. Raptor X was used to predict 3D structure of the MHC class I of Equas Caballus ELA-A3 haplotype (NP_001116853.1). These structures were further prepared for running docking in Patch Dock server [45] and visualized using Chimera 1.8. [43]

3. Results

3.1. Phylogenetic Evolution

Phylogenetic tree demonstrated the evolutionary divergence within the retrieved sequences of the glycoprotein. As shown in Figure 1 retrieved strains showed molecular divergence in their common ancestors.

3.2. Sequences Alignment

Sequence alignment of all retrieved strains was performed using ClustalW that presented by Bioedit software. As shown in Figure 2, the retrieved sequences demonstrated conservancy when sequences were aligned. The conserved regions were recognized by identity of amino acid sequences among the retrieved sequences.

3.3. B-cell Epitope Prediction

As shown in Figure 3, BepiPred linear epitopes prediction, Emini surface accessibility and kolaskar and tongaonker antigenicity tools were used for prediction of B cells epitopes with thresholds of 0.031, 1.000 and 1.035, respectively. According to the criteria of B cell prediction tools; Bepipred linear epitope prediction tools proposed 32 epitopes as a linear epitopes. Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods proposed 25 and 13 epitopes, respectively. However ten epitopes only has passed the three criteria of B cell prediction tools. Table 2 demonstrated all these epitopes that predicted by the B cell tools. Among these epitopes; six epitopes were proposed as B cell epitopes since they got high scores in the three tools. The 3D structures of these six epitopes in the glycoprotein were provided in Figure 4.

3.4. MHC Class I Binding Predictions

IEDB MHCI epitope prediction tool generated 10 unique binding epitopes from the Glycoprotein having affinity values less or equal 300 nM that bound to highest number of MHCI alleles. These epitopes were demonstrated in Table 3. From these ten epitopes; four epitopes namely $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, $_{189}$GESVCSQLF$_{197}$ and $_{108}$CLEAIKAYK$_{116}$ were proposed as MHC-1 epitopes since they bound to high number of alleles with less IC50 and percentile rank. The 3D structures of these epitopes in the glycoprotein were provided in Figure 5 and Figure 6.

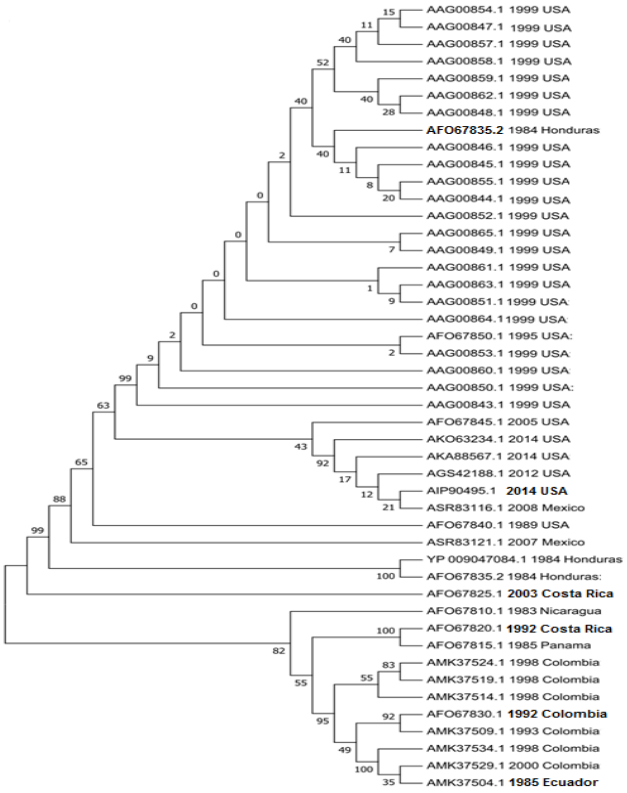

Figure 1. Evolutionary divergence analysis of capsid protein of the HEV. The retrieved strains showed divergence in their common ancestors

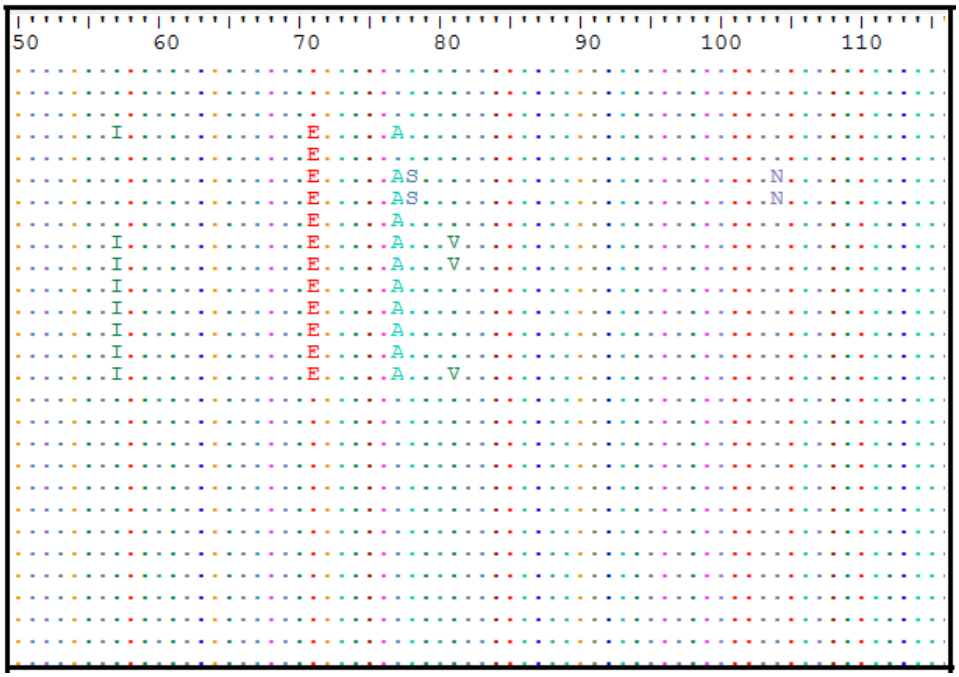

Figure 2. Multiple sequence alignment (MSA) of the retrieved strains using Bioedit software and ClustalW. Dots indicated the conservancy and letters showed the alteration in amino acid

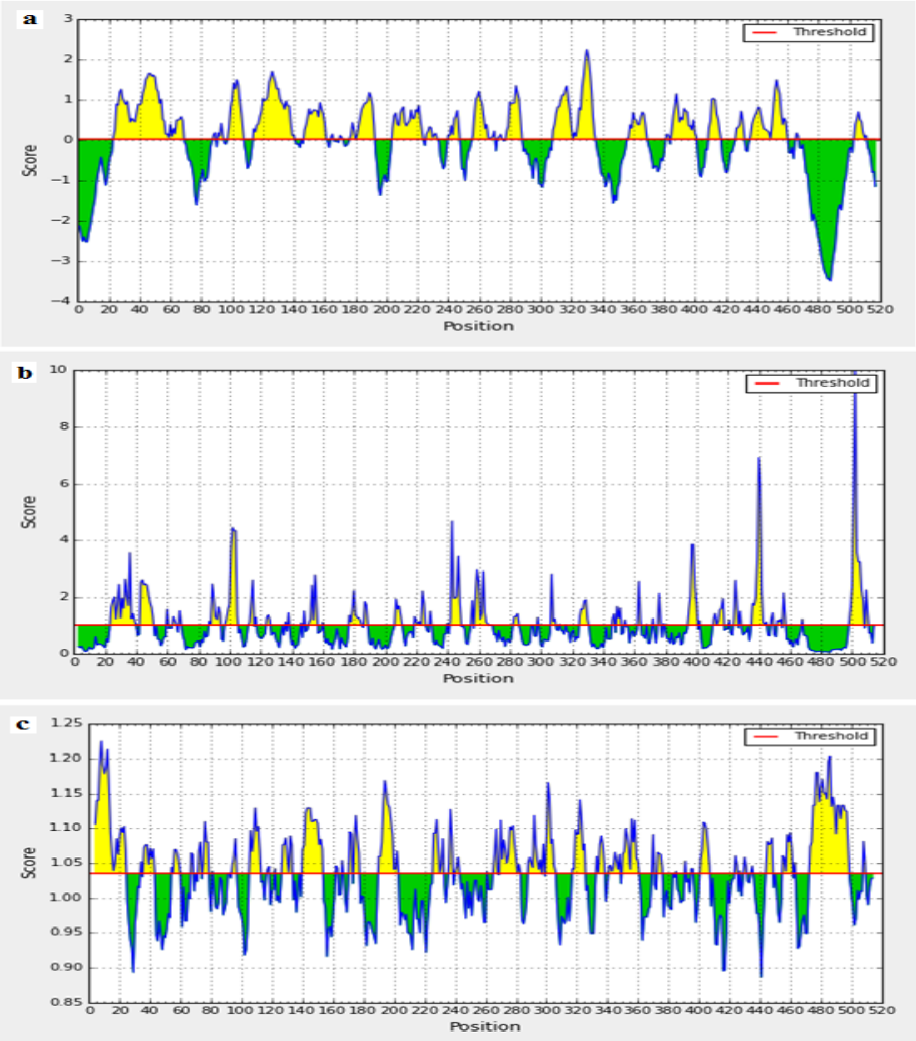

Figure 3. Prediction of B-cell epitopes by different IEDB scales (**a**- Bepipred linear epitope prediction threshold 0.031, **b**- Emini surface accessibility threshold 1.000, **c**- Kolaskar and Tongaonkar antigenicity prediction threshold 1.035). Regions above threshold (red line) are proposed to be a part of B cell epitope while regions below the threshold (red line) were not

Table 2. B cell predicted epitopes. The table demonstrated the predicted conserved epitopes with their surface accessibility score (a) and antigenicity score (b)

Peptide	Start	End	Length	Emini score 1.000	Antigenicity score 1.035
PQHT	23	26	4	2.102	1.023
GDWK	28	31	4	1.389	0.891
PKYI	91	94	4	1.357	1.077
DCDL	272	275	4	0.493	1.099
AGEP	213	216	4	1.07	0.963
SAYKDG	114	118	5	1.655	0.979
DPDLD	257	261	5	1.879	0.982
DLPHI	266	270	5	0.643	1.087
DLPH*	266	269	4	1.158	1.071
TGEVD	409	413	5	0.97	0.977
#PIYKS	505	509	5	1.44	1.064
GPKNPG	326	331	6	1.871	0.93
IPRMEG	360	365	6	0.895	0.94
HPHIEA	426	432	6	0.872	1.057
#HPHIE*	427	431	5	1.099	1.055
LKKDDT	437	442	6	3.298	0.959
YYGDTG	447	452	6	1.44	0.974
GLPETG	215	220	6	0.775	0.97
SIHNEEP	97	103	7	1.878	0.973
FFSDSEE	204	210	7	1.328	0.968
PGEHATD	282	288	7	1.729	0.962
#SKNPVEL	454	460	7	1.391	1.038
#CRKPGYKL	240	247	8	1.295	1.062
FNPGFPPQSCGYG	121	133	13	0.432	1.029
PHSVKVDEYTGEWID	147	161	15	1.424	1.02
PHSVK*	147	151	5	1.324	1.099
#PHSVKVDEY*	147	155	9	1.742	1.084
EIGPNGVLKTKQGYK	387	401	15	1.863	0.998
#VLKTKQGYK*	393	401	9	2.348	1.042
TVHNSTKWFTSSDGESV	176	192	17	1.286	0.996
YNYCPTSADKNSHGTQTGIP	37	56	20	2.671	1.002
YNYCPTSADK*	37	46	10	2.107	1.035

*Peptide after being shortened
#B cell proposed epitopes.

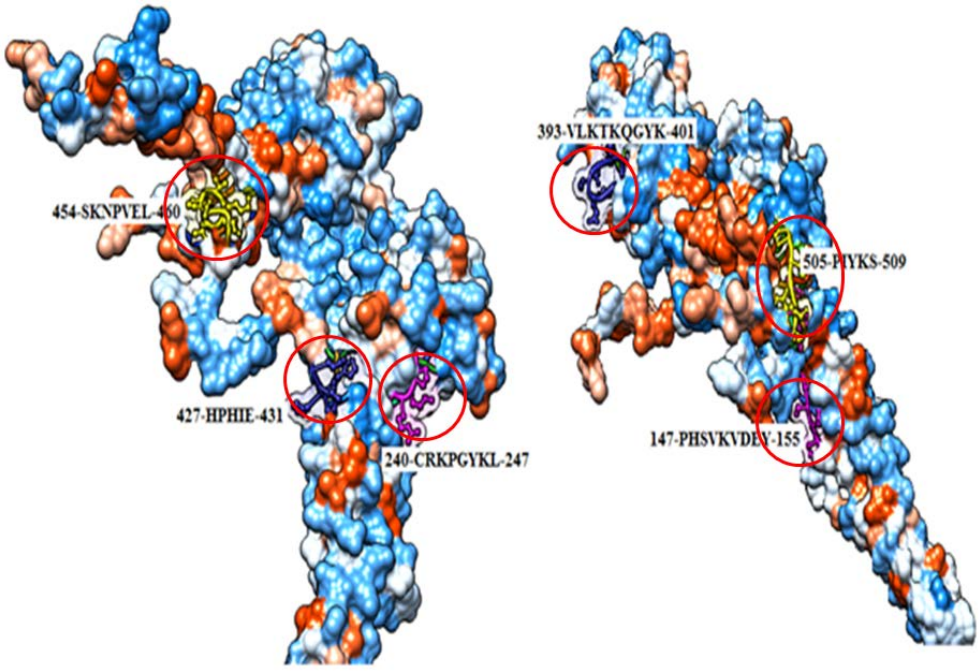

Figure 4. Structural positions of the B cell epitopes of glycoprotein of New Jersey Vesicular stomatitis virus. The position of the six epitopes was shown encircled. The figure showed the positions of these epitopes from different sites of view of the same protein

Table 3. List of best epitopes that had binding affinity with the human MHC class I alleles

Peptide	Start	End	Allele	ic50	Percentile
FRWYGPKYI#	86	94	HLA-B*27:05	112.67	0.3
			HLA-C*06:02	24.67	0.1
			HLA-C*07:01	94.4	0.1
			HLA-C*07:02	160.32	0.3
			HLA-C*12:03	115.43	0.2
FTSSDGESV#	184	192	HLA-A*02:06	58.38	0.7
			HLA-A*68:02	25.21	0.6
			HLA-C*03:03	238.64	1
			HLA-C*12:03	71.14	0.2
GESVCSQLF#	189	197	HLA-B*40:01	58.6	0.2
			HLA-B*40:02	271.47	0.4
			HLA-B*44:02	8.96	0.1
			HLA-B*44:03	30.43	0.1
CLEAIKAYK#	108	116	HLA-A*03:01	253.54	0.4
			HLA-A*31:01	245.93	0.6
			HLA-A*68:01	168.01	1.3
ETVHNSTKW	175	183	HLA-B*57:01	226.23	0.3
			HLA-B*58:01	36.81	0.3
GICKMPFCR	233	241	HLA-A*11:01	223.57	0.4
			HLA-A*31:01	102.98	0.5
GPKYITHSI	90	98	HLA-B*07:02	36	0.2
			HLA-B*08:01	297.85	0.5
GTRIVRQLW	369	377	HLA-B*57:01	13.31	0.2
			HLA-B*58:01	194.54	0.5
ISLISDVER	289	297	HLA-A*31:01	107.56	0.5
			HLA-A*68:01	81.93	0.6
KVDEYTGEW	151	159	HLA-B*58:01	29.97	0.3
			HLA-C*05:01	131.16	0.4

MHC-1 proposed epitopes.

Table 4. List of the best four epitopes that had binding affinity with the human MHC class II alleles

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
IEIVFPQHT	SPILGKIEIVFPQHT	12	26	HLA-DRB1*01:01	134.4	34.49
				HLA-DRB1*07:01	360.8	28.69
				HLA-DRB1*13:02	472.4	16.82
				HLA-DRB1*15:01	281.9	21.11
				HLA-DRB4*01:01	55.5	4.2
	PILGKIEIVFPQHTT	13	27	HLA-DRB1*01:01	64.1	24.31
				HLA-DRB1*07:01	376.8	29.3
				HLA-DRB1*11:01	749.7	36.58
				HLA-DRB1*13:02	467.1	16.71
	PILGKIEIVFPQHTT			HLA-DRB1*15:01	245.5	19.37
				HLA-DRB4*01:01	66	5.09
	ILGKIEIVFPQHTTG	14	28	HLA-DQA1*05:01/DQB1*02:01	762	16.34
				HLA-DRB1*01:01	41.4	19.03
				HLA-DRB1*07:01	537.4	34.35
				HLA-DRB1*11:01	371.8	27.32
				HLA-DRB1*13:02	655.5	20.4
				HLA-DRB1*15:01	263.1	20.23
				HLA-DRB4*01:01	66.6	5.14
	LGKIEIVFPQHTTGD	15	29	HLA-DRB1*01:01	42.1	19.22
				HLA-DRB1*04:05	501.2	29.32
				HLA-DRB1*07:01	736.6	39.24
				HLA-DRB1*09:01	999.8	38.96
				HLA-DRB1*11:01	313.9	25.3
				HLA-DRB1*13:02	662.1	20.51
				HLA-DRB1*15:01	372.2	24.85
				HLA-DRB4*01:01	65.1	5.01

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
	GKIEIVFPQHTTGDW	16	30	HLA-DRB1*01:01	125.9	33.51
				HLA-DRB1*08:02	220	4.93
				HLA-DRB1*11:01	378	27.51
				HLA-DRB1*13:02	887.2	24.24
				HLA-DRB1*15:01	454.3	27.66
				HLA-DRB4*01:01	64.2	4.93
	KIEIVFPQHTTGDWK	17	31	HLA-DRB1*01:01	248.1	44.72
				HLA-DRB1*08:02	417.5	10.12
				HLA-DRB1*15:01	576.6	31.23
				HLA-DRB4*01:01	57.4	4.37
	IEIVFPQHTTGDWKR	18	32	HLA-DRB1*01:01	657.1	61.01
				HLA-DRB4*01:01	79.8	6.21
FRWYGPKYI	WMTTCDFRWYGPKYI	80	94	HLA-DPA1*01:03/DPB1*02:01	931.6	29.43
				HLA-DQA1*05:01/DQB1*03:01	312.4	28.61
				HLA-DRB1*01:01	541	57.99
				HLA-DRB1*07:01	11.2	1.93
				HLA-DRB1*09:01	33.9	1.87
				HLA-DRB1*13:02	377	14.69
				HLA-DRB1*15:01	28.3	2.54
				HLA-DRB3*01:01	57.3	3.08
				HLA-DRB5*01:01	12.2	2.99
	MTTCDFRWYGPKYIT	81	95	HLA-DPA1*01:03/DPB1*02:01	568.6	22.98
				HLA-DQA1*05:01/DQB1*03:01	227.3	24.21
				HLA-DRB1*01:01	225.7	43.01
				HLA-DRB1*07:01	12.9	2.32
				HLA-DRB1*09:01	29.5	1.52
				HLA-DRB1*13:02	379.4	14.74
				HLA-DRB1*15:01	25	2.13
				HLA-DRB3*01:01	57.7	3.09
				HLA-DRB5*01:01	9.5	2.22
	TTCDFRWYGPKYITH	82	96	HLA-DPA1*01:03/DPB1*02:01	211.3	13.31
				HLA-DQA1*05:01/DQB1*03:01	220.9	23.84
				HLA-DRB1*01:01	97.8	29.88
				HLA-DRB1*07:01	16.3	3.06
	TTCDFRWYGPKYITH	82	96	HLA-DRB1*09:01	26.4	1.29
				HLA-DRB1*11:01	612.8	33.73
				HLA-DRB1*13:02	356.1	14.18
				HLA-DRB1*15:01	21.5	1.71
				HLA-DRB3*01:01	61.8	3.25
				HLA-DRB5*01:01	7.3	1.55
	TCDFRWYGPKYITHS	83	97	HLA-DPA1*01/DPB1*04:01	952.3	21.24
				HLA-DQA1*05:01/DQB1*03:01	194	22.19
				HLA-DRB1*01:01	45.7	20.18
				HLA-DRB1*07:01	21.1	4.07
	TCDFRWYGPKYITHS	83	97	HLA-DRB1*09:01	26.3	1.28
				HLA-DRB1*11:01	412.4	28.59
				HLA-DRB1*13:02	365.8	14.42
				HLA-DRB1*15:01	25.2	2.16
				HLA-DRB3*01:01	70.3	3.56
				HLA-DRB5*01:01	6.3	1.24
	CDFRWYGPKYITHSI	84	98	HLA-DQA1*05:01/DQB1*03:01	202	22.71
				HLA-DRB1*01:01	104.1	30.75
				HLA-DRB1*07:01	27.7	5.3
				HLA-DRB1*09:01	32.7	1.78
				HLA-DRB1*11:01	424.1	28.95
				HLA-DRB1*13:02	554.9	18.51
				HLA-DRB1*15:01	30.2	2.76
				HLA-DRB3*01:01	119.8	5.02
				HLA-DRB5*01:01	8.1	1.8

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
	DFRWYGPKYITHSIH	85	99	HLA-DPA1*01/DPB1*04:01	855.4	20.05
				HLA-DQA1*05:01/DQB1*03:01	214.3	23.46
				HLA-DRB1*01:01	190	40.02
				HLA-DRB1*07:01	33.5	6.27
				HLA-DRB1*09:01	41	2.49
				HLA-DRB1*13:02	888.7	24.27
				HLA-DRB1*15:01	36.2	3.48
				HLA-DRB3*01:01	200.9	6.88
				HLA-DRB5*01:01	10.9	2.63
	FRWYGPKYITHSIHN	86	100	HLA-DPA1*01/DPB1*04:01	967.7	21.43
				HLA-DPA1*01:03/DPB1*02:01	439.7	20.07
				HLA-DQA1*05:01/DQB1*03:01	239	24.87
				HLA-DRB1*01:01	188.9	39.93
				HLA-DRB1*09:01	66.5	4.5
				HLA-DRB1*15:01	75.5	7.72
				HLA-DRB3*01:01	339.7	9.36
				HLA-DRB5*01:01	12.4	3.03
FTSSDGESV	HNSTKWFTSSDGESV	178	192	HLA-DQA1*05:01/DQB1*03:01	752.2	42.78
				HLA-DRB1*04:05	469.4	28.29
				HLA-DRB1*07:01	64.6	10.5
				HLA-DRB1*09:01	196.4	12.75
				HLA-DQA1*05:01/DQB1*03:01	609	39.1
				HLA-DRB1*01:01	841.7	64.73
				HLA-DRB1*04:05	475.1	28.47
				HLA-DRB1*07:01	76	11.79
				HLA-DRB1*09:01	149.2	10.06
	STKWFTSSDGESVCS	180	194	HLA-DQA1*05:01/DQB1*03:01	517.8	36.34
				HLA-DRB1*01:01	564.7	58.66
				HLA-DRB1*04:01	537.4	30.16
				HLA-DRB1*04:05	541.3	30.56
				HLA-DRB1*07:01	103.9	14.53
				HLA-DRB1*09:01	115.4	7.94
				HLA-DRB3*01:01	916.3	16.91
	TKWFTSSDGESVCSQ	181	195	HLA-DQA1*05:01/DQB1*03:01	481.9	35.18
				HLA-DRB1*01:01	361	51.34
				HLA-DRB1*04:01	587.4	31.84
				HLA-DRB1*04:05	650.9	33.61
				HLA-DRB1*07:01	159.9	18.94
				HLA-DRB1*09:01	88.1	6.11
				HLA-DRB3*01:01	868.4	16.36
				HLA-DRB5*01:01	962.3	42.63
	KWFTSSDGESVCSQL	182	196	HLA-DQA1*05:01/DQB1*03:01	482.1	35.18
				HLA-DRB1*01:01	547.5	58.18
				HLA-DRB1*07:01	254.7	24.23
				HLA-DRB1*09:01	130.4	8.89
	WFTSSDGESVCSQLF	183	197	HLA-DQA1*05:01/DQB1*03:01	468	34.71
				HLA-DRB1*01:01	811.3	64.18
				HLA-DRB1*04:05	784.2	36.92
				HLA-DRB1*07:01	285.3	25.62
				HLA-DRB1*09:01	239.7	14.93
	FTSSDGESVCSQLFT	184	198	HLA-DQA1*05:01/DQB1*03:01	575.4	38.11
				HLA-DRB1*01:01	849.3	64.86
				HLA-DRB1*07:01	453.4	31.89
				HLA-DRB1*09:01	403	22.07

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
LKNDLWFQI	RKPGYKLKNDLWFQI	241	255	HLA-DPA1*01/DPB1*04:01	384.7	12.77
				HLA-DPA1*01:03/DPB1*02:01	109.9	8.87
				HLA-DPA1*02:01/DPB1*01:01	57.5	6.14
				HLA-DPA1*03:01/DPB1*04:02	171.5	13.54
				HLA-DRB1*04:05	364.5	24.47
				HLA-DRB1*13:02	897.7	24.4
				HLA-DRB3*01:01	52.6	2.9
				HLA-DRB5*01:01	250.1	24.5
	KPGYKLKNDLWFQIT	242	256	HLA-DPA1*01/DPB1*04:01	305.3	11.11
				HLA-DPA1*01:03/DPB1*02:01	94.8	8.07
				HLA-DPA1*02:01/DPB1*01:01	46.5	4.87
				HLA-DPA1*03:01/DPB1*04:02	99.8	9.65
				HLA-DRB1*04:05	384.5	25.26
				HLA-DRB1*08:02	947.6	21.52
				HLA-DRB1*13:02	727.6	21.67
				HLA-DRB3*01:01	50.4	2.8
				HLA-DRB5*01:01	210.2	22.53
	PGYKLKNDLWFQITD	243	257	HLA-DPA1*01/DPB1*04:01	323.8	11.51
				HLA-DPA1*01:03/DPB1*02:01	109.1	8.83
				HLA-DPA1*02:01/DPB1*01:01	45.3	4.73
				HLA-DPA1*03:01/DPB1*04:02	86.2	8.74
				HLA-DRB1*04:05	404.1	25.99
				HLA-DRB1*08:02	783.4	18.32
				HLA-DRB1*09:01	905.5	36.8
				HLA-DRB1*13:02	680.6	20.83
				HLA-DRB3*01:01	45.8	2.61
				HLA-DRB5*01:01	267.2	25.25
	GYKLKNDLWFQITDP	244	258	HLA-DPA1*01/DPB1*04:01	326.4	11.57
				HLA-DPA1*01:03/DPB1*02:01	115.2	9.14
				HLA-DPA1*02:01/DPB1*01:01	50	5.28
				HLA-DPA1*03:01/DPB1*04:02	84.2	8.59
				HLA-DRB1*04:05	506.3	29.48
				HLA-DRB1*08:02	642.7	15.39
				HLA-DRB1*09:01	845.3	35.38
				HLA-DRB1*13:02	766	22.31
				HLA-DRB3*01:01	50.4	2.8
				HLA-DRB5*01:01	346.3	28.31
	YKLKNDLWFQITDPD	245	259	HLA-DPA1*01/DPB1*04:01	360.2	12.27
				HLA-DPA1*01:03/DPB1*02:01	125	9.64
				HLA-DPA1*02:01/DPB1*01:01	50.7	5.36
				HLA-DPA1*03:01/DPB1*04:02	98.4	9.56
				HLA-DRB1*08:02	760.2	17.89
				HLA-DRB1*13:02	993.7	25.82
				HLA-DRB3*01:01	102.5	4.55
				HLA-DRB5*01:01	674.7	37.26
	KLKNDLWFQITDPDL	246	260	HLA-DPA1*01/DPB1*04:01	584.8	16.28
				HLA-DPA1*01:03/DPB1*02:01	245.5	14.52
				HLA-DPA1*02:01/DPB1*01:01	73.8	7.92
				HLA-DPA1*03:01/DPB1*04:02	193.5	14.52
				HLA-DRB1*08:02	918.9	21.01
				HLA-DRB3*01:01	228	7.41
	LKNDLWFQITDPDLD	247	261	HLA-DPA1*01/DPB1*04:01	773.7	18.98
				HLA-DPA1*01:03/DPB1*02:01	453.9	20.42
				HLA-DPA1*02:01/DPB1*01:01	150.6	14.44
				HLA-DPA1*03:01/DPB1*04:02	359.6	20.16
				HLA-DRB3*01:01	436.7	10.86

3.5. MHC class II binding predictions

Peptides binding to MHC class II molecules were evaluating with the IEDB MHC-II prediction tools using human alleles. Several methods were used for analysis of MHC-II epitopes binding grooves. However the NN- align is important for instantaneous identification of the MHC class II binding core epitope. In this study, several core peptides were predicted to interact with considerable number of MHCII alleles. However four epitopes namely $_{241}$LKNDLWFQI$_{255}$, $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, and $_{18}$IEIVFPQHT$_{26}$ demonstrated great binding interaction with 82, 78 and 44 and 39 MHC-II alleles, respectively. These four epitopes and their interacting alleles were provided in Table 4. The epitopes $_{86}$FRWYGPKYI$_{94}$ and $_{184}$FTSSDGESV$_{192}$ were found interacting in both MHC-I and MHC-II alleles. Thus their positions in the 3D structures of glycoprotein was depicted in Figure 5 while the 3D structures of $_{18}$IEIVFPQHT$_{26}$ and $_{241}$LKNDLWFQI$_{255}$ in the glycoprotein were depicted in Figure 6.

3.6 Docking of the Selected Epitopes with MHC Class 1 Alleles

Binding models of the best probable epitopes ($_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, $_{189}$-GESVCSQLF$_{-197}$ and $_{108}$-CLEAIKAYK$_{-116}$) to the equine ELA-A3 molecule was observed using Patch dock. As demonstrated in Table 5 lowest binding energy (kcal/mol) was selected to predict probable CTL epitopes based on the score of global energy and attractive VDW in kcal/mol unit of docked molecules. Docking of $_{86}$FRWYGPKYI$_{94}$ and $_{184}$FTSSDGESV$_{192}$ with ELA-A3 allele produced -22.07kcal/mol and -70.22kcal/mol global energy respectively. This indicated the strong binding affinity between the ligand and the receptor. Moreover, $_{189}$-GESVCSQLF$_{-197}$ and $_{108}$-CLEAIKAYK$_{-116}$ epitopes demonstrated favorable binding affinity with ELA-A3 allele with -23.72kcal/mol and 1.34kcal/mol global energy, respectively. Generally the docked molecules showed different binding site for ELA-A3 allele (Figure 7 and Figure 8). These results revealed that the docked epitopes could induce the humoral and cellular immunity responses.

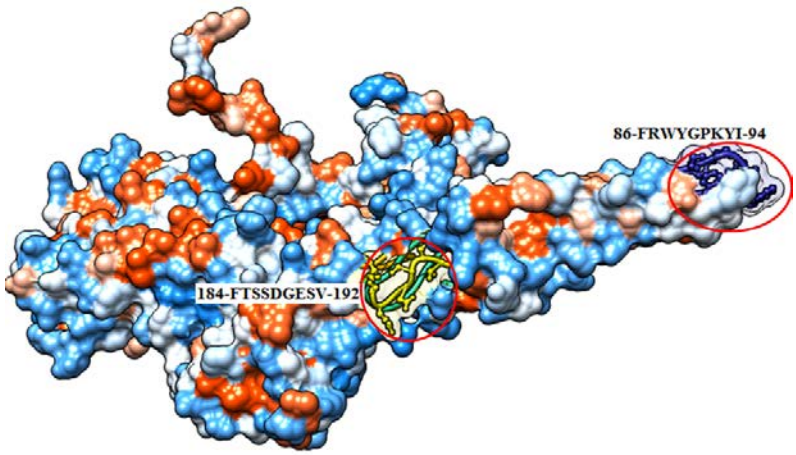

Figure 5. Structural position of the two epitopes of glycoprotein of New Jersey Vesicular stomatitis virus that interacted with both MHC-I & MHC-II alleles. Also these two epitopes were docked with equine haplotype molecule

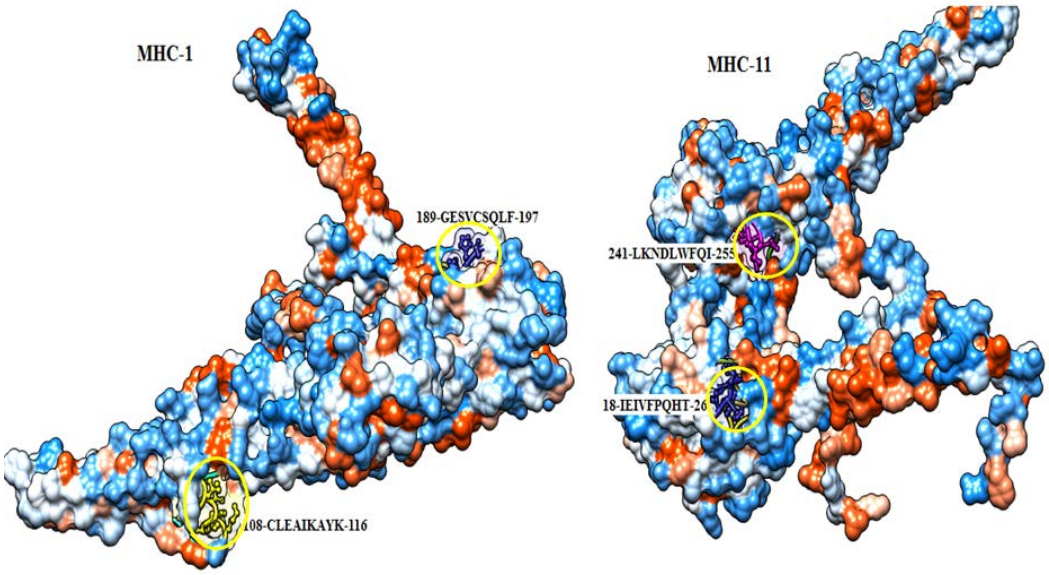

Figure 6. Structural positions of the other two promising T cell epitopes of glycoprotein of New Jersey Vesicular stomatitis virus. The position of the epitopes was shown in dark blue, purple and yellow colors. The figure showed the positions of these epitopes from different sites of view of the same protein

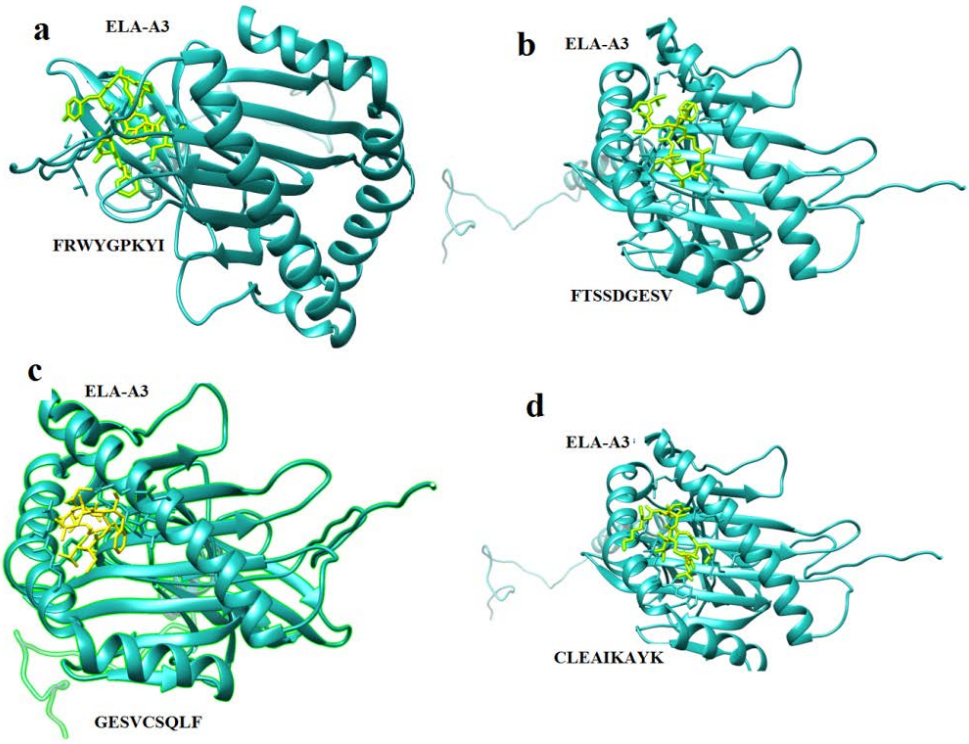

Figure 7. Visualization of the 3D structure of ELA-A3 equine allele (in green color) using chimera visualization tool. The **a, b, c and d** are the docked epitopes $_{86}$FRWYGPKYI$_{94}$, $_{184}$FTSSDGESV$_{192}$, $_{189}$GESVCSQLF$_{197}$ and $_{108}$CLEAIKAYK$_{116}$, respectively, (shown in yellow color) that proposed for CTL for docking with ELA-A3 equine allele

Table 5. The binding energy and Attractive VDW scores for the proposed epitopes with ELA-A3 equine allele using PatchDock server

Epitopes	Start	End	Receptor	Binding Energy Score (kcal/mol)
FTSSDGESV	184	192	ELA-A3	-70.22
GESVCSQLF	189	197	ELA-A3	-23.72
FRWYGPKYI	86	94	ELA-A3	-22.07
CLEAIKAYK	108	116	ELA-A3	-1.34

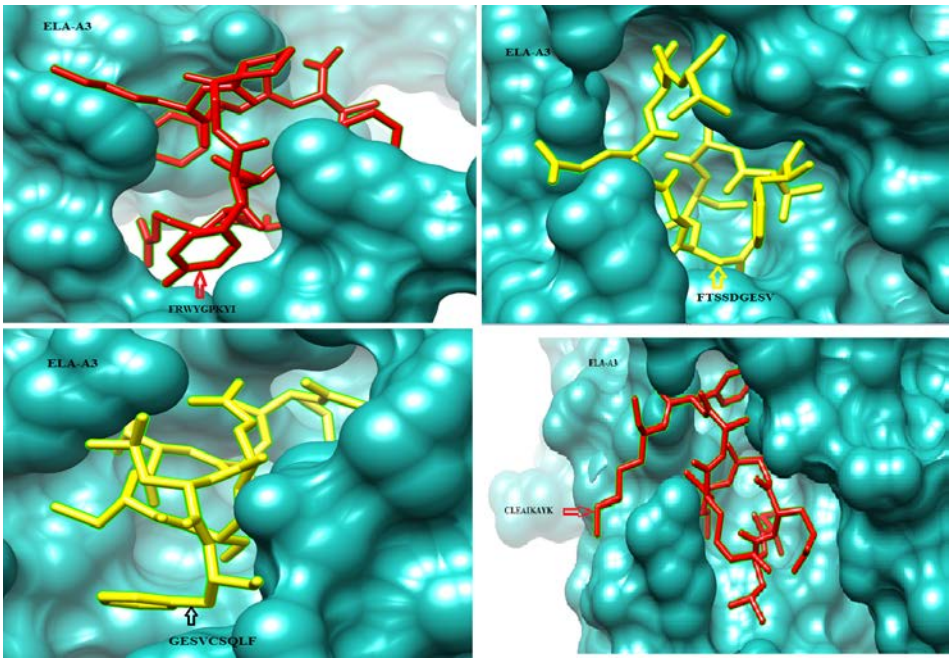

Figure 8. Visualization of PatchDock Molecular docking of MHCI proposed epitopes and ELA-A3 equine allele receptors using UCSF-Chimera visualization tool. Receptors (ELA-A3 allele) represented by rounded ribbon structure green colour while CTL epitopes represent by yellow and red colors

4. Discussion

Vesicular stomatitis (VS) is contagious disease caused by Vesicular stomatitis virus (VSV) often causes a mortality rate exceeding 90% in horse. Vaccination is effective method of preventing Vesicular stomatitis (VS) such as inactivated preparation vaccine, live attenuated vaccine, and DNA mediated vaccine. But all these vaccine available have not performed successfully. Therefore, in this study we aim to predict peptide vaccine for the Vesicular stomatitis New Jersey virus of its glycoprotein as an immunogen to stimulate protective immune response. Peptide- based vaccine has good desirable immune response and a minimal immunological side effect. There are many peptide vaccines under development of designing vaccine and therapeutic for most infectious diseases such as influenza virus, cancer, Ebola virus, and other [46-50].

In this study the selected peptides that could be recognized by B cell and T cell epitope. For B cell epitope prediction; eight epitopes **PKYI**, **VLKTKQGYK**, **PHSVKVDEY**, **SKNPVEL**, **CRKPGYKL**, **HPHIE**, **PIYKS**, and **DLPH** got scores above the threshold of Emini and antigenicity, therefore that capable of inducing the desired immune response as B cell epitope. For MHC I and II binding prediction used human alleles in IEDB T-cell prediction tools since it showed similarity, because haven't entered equine alleles as data [40,51,52].

There were two epitopes "**FRWYGPKYI**, **FTSSDGESV**" interacted with the highest numbers of MHC class I alleles and have high binging affinity and the lowest binding energy to equine MHC class I molecule (ELA-A3) haplotype in the structural level. **FRWYGPKYI** epitope was found to bind 5 MHCI Alleles; HLA-B*27:05, HLA-C*06:02, HLA-C*07:01, HLA-C*07:02, HLA-C*12:03 and FTSSDGESV epitope was found to bind 4 MHC II Alleles HLA-A*02:06, HLA-A*68:02, HLA-C*03:03, HLA-C*12:03. While in MHC II prediction, five most potential epitopes were chosen on the basis of highest number of MHC II alleles "**IEIVFPQHT**, **FRWYGPKYI**, **FTSSDGESV**, **LKNDLWFQI**, and **LISDVERIL**. This study proposed an interesting T cell epitope (**FRWYGPKYI**, **FTSSDGESV**) that have very strong binding affinity to both MHC1 and MHC11 alleles. So this epitope based vaccine would be able to elicit both humeral and cell mediated immunity. To our knowledge there is no epitope based vaccine for the Vesicular stomatitis New Jersey Virus (VSV-NJ) using immunoinformatics approach.

5. Conclusion

This study predicted novel B-cells and T-cell epitopes from the glycoprotein of Vesicular stomatitis New Jersey Virus strains. The proposed epitopes showed conservancy rates and demonstrated potentiality as based peptide vaccine for application in the control of Vesicular stomatitis New Jersey Virus infection. The use of such vaccines will likely reduce the challenges associated with live attenuated vaccines and allow broad coverage of the target Vesicular stomatitis New Jersey Virus strains. Accordingly Immunoinformatic techniques focused on immune response would avoid undesirable epitopes and reducing costs for designing of new vaccines and therapies.

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Data Availability

The [glycoprotein of the retrieved strains was from the NCBI. Also analysis of the epitopes was in IEDB] data used to support the findings of this study are included within the article.

Conflict of Interests

The authors declared that they have no conflict of interests regarding the publication of this paper.

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References

[1] Alvarado JF, Dolz G, Herrero MV, McCluskey B, Salman M. Comparison of the serum neutralization test and a competitive enzyme-linked immunosorbent assay for the detection of antibodies to vesicular stomatitis virus New Jersey and vesicular stomatitis virus Indiana. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2002; 14(3): 240-2.

[2] Cornish TE, Stallknecht DE, Brown CC, Seal BS, Howerth EW. Pathogenesis of experimental vesicular stomatitis virus (New Jersey serotype) infection in the deer mouse (Peromyscus maniculatus). Veterinary pathology. 2001; 38(4): 396-406.

[3] Reis JL, Rodriguez LL, Mead DG, Smoliga G, Brown CC. Lesion development and replication kinetics during early infection in cattle inoculated with Vesicular stomatitis New Jersey virus via scarification and black fly (Simulium vittatum) bite. Veterinary pathology. 2011; 48(3): 547-57.

[4] Smith PF, Howerth EW, Carter D, Gray EW, Noblet R, Smoliga G, et al. Domestic cattle as a non-conventional amplifying host of vesicular stomatitis New Jersey virus. Medical and veterinary entomology. 2011; 25(2): 184-91.

[5] Lee HS, Heo EJ, Jeoung HY, Ko HR, Kweon CH, Youn HJ, et al. Enzyme-linked immunosorbent assay using glycoprotein and monoclonal antibody for detecting antibodies to vesicular stomatitis virus serotype New Jersey. Clinical and vaccine immunology : CVI. 2009; 16(5): 667-71.

[6] Scherer CF, O'Donnell V, Golde WT, Gregg D, Estes DM, Rodriguez LL. Vesicular stomatitis New Jersey virus (VSNJV) infects keratinocytes and is restricted to lesion sites and local lymph nodes in the bovine, a natural host. Veterinary research. 2007; 38(3): 375-90.

[7] Arshed MJ, Magnuson RJ, Triantis J, Abubakar M, Van Campen H, Salman M. Comparison of RNA extraction methods to augment the sensitivity for the differentiation of vesicular stomatitis virus Indiana1 and New Jersey. Journal of clinical laboratory analysis. 2011; 25(2): 95-9.

[8] Fowler VL, Howson EL, Madi M, Mioulet V, Caiusi C, Pauszek SJ, et al. Development of a reverse transcription loop-mediated

isothermal amplification assay for the detection of vesicular stomatitis New Jersey virus: Use of rapid molecular assays to differentiate between vesicular disease viruses. Journal of virological methods. 2016; 234: 123-31.

[9] Rainwater-Lovett K, Pauszek SJ, Kelley WN, Rodriguez LL. Molecular epidemiology of vesicular stomatitis New Jersey virus from the 2004-2005 US outbreak indicates a common origin with Mexican strains. The Journal of general virology. 2007; 88(Pt 7): 2042-51.

[10] Smith PF, Howerth EW, Carter D, Gray EW, Noblet R, Berghaus RD, et al. Host predilection and transmissibility of vesicular stomatitis New Jersey virus strains in domestic cattle (Bos taurus) and swine (Sus scrofa). BMC veterinary research. 2012; 8: 183.

[11] Killmaster LF, Stallknecht DE, Howerth EW, Moulton JK, Smith PF, Mead DG. Apparent disappearance of Vesicular Stomatitis New Jersey Virus from Ossabaw Island, Georgia. Vector borne and zoonotic diseases. 2011; 11(5): 559-65.

[12] Rasmussen TB, Uttenthal A, Fernandez J, Storgaard T. Quantitative multiplex assay for simultaneous detection and identification of Indiana and New Jersey serotypes of vesicular stomatitis virus. Journal of clinical microbiology. 2005; 43(1): 356-62.

[13] Velazquez-Salinas L, Isa P, Pauszek SJ, Rodriguez LL. Complete Genome Sequences of Two Vesicular Stomatitis Virus Isolates Collected in Mexico. Genome announcements. 2017; 5(37).

[14] Martinez I, Barrera JC, Rodriguez LL, Wertz GW. Recombinant vesicular stomatitis (Indiana) virus expressing New Jersey and Indiana glycoproteins induces neutralizing antibodies to each serotype in swine, a natural host. Vaccine. 2004; 22(29-30): 4035-43.

[15] Wu K, Kim GN, Kang CY. Expression and processing of human immunodeficiency virus type 1 gp160 using the vesicular stomatitis virus New Jersey serotype vector system. The Journal of general virology. 2009; 90(Pt 5): 1135-40.

[16] Georgel P, Jiang Z, Kunz S, Janssen E, Mols J, Hoebe K, et al. Vesicular stomatitis virus glycoprotein G activates a specific antiviral Toll-like receptor 4-dependent pathway. Virology. 2007; 362(2): 304-13.

[17] Kim IS, Jenni S, Stanifer ML, Roth E, Whelan SP, van Oijen AM, et al. Mechanism of membrane fusion induced by vesicular stomatitis virus G protein. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114(1): E28-E36.

[18] Bachmann MF, Kundig TM, Kalberer CP, Hengartner H, Zinkernagel RM. Formalin inactivation of vesicular stomatitis virus impairs T-cell- but not T-help-independent B-cell responses. Journal of virology. 1993; 67(7): 3917-22.

[19] House JA HC, Dubourget P, Lombard M. Protective immunity in cattle vaccinated with a commercial scale, inactivated, bivalent vesicular stomatitis vaccine. Vaccine. 2003: 1932-7.

[20] J.D. Cantlon PWG, R.A. Bowen. Immune responses in mice, cattle and horses to a DNA vaccine for vesicular stomatitis. Vaccine. 2000; 18: 2368-74.

[21] Flanagan EB, Zamparo JM, Ball LA, Rodriguez LL, Wertz GW. Rearrangement of the genes of vesicular stomatitis virus eliminates clinical disease in the natural host: new strategy for vaccine development. Journal of virology. 2001; 75(13): 6107-14.

[22] Ruth E. Soria-Guerra RN-G, Dania O. Govea-Alonso, Sergio Rosales-Mendoza. An overview of bioinformatics tools for epitope prediction: Implications on vaccine development. Journal of Biomedical Informatics. 2014:1-9.

[23] Doytchinova APaI. T-cell epitope vaccine design by immunoinformatics. Open Biology. 2013:1-13.

[24] Bette Korber ML, Karina Yusim. Immunoinformatics Comes of Age. PLoS Computational Biology. 2006; 2(6): 484-92.

[25] Jonathan M. Gershoni AR-B, Dror D. Siman-Tov, Natalia Tarnovitski Freund and, Weiss Y. Epitope Mapping The First Step in Developing Epitope-Based Vaccines. Biodrugs. 2007: 145-56.

[26] Usman Sumo Friend Tambunan FRPS, Arli Aditya Parikesit and Djati Kerami. Vaccine Design for H5N1 Based on B- and T-cell Epitope Predictions. Bioinformatics and Biology Insights. 2016: 27-35.

[27] Perla Carlos V, Sébastien Holbert, Felipe Ascencio, Kris Huygen, Gracia Gomez-Anduro, MaximeBranger, MarthaReyes-Becerril, Carlos Angulo. In silico epitope analysis of unique and membrane associated proteins from Mycobacteriumavium subsp. paratuberculosis for immunogenicity and vaccine evaluation. Journal of Theoretical Biology. 2015:1-9.

[28] Malaz Abdelbagi TH, Mohammed Shihabeldin, Sanaa Bashir, Elkhaleel Ahmed, Elmoez Mohamed, Shawgi Hafiz, Abdah Abdelmonim, Tassneem Hamid, Shimaa Awad, Ahmed Hamdi, Khoubieb Ali and Mohammed A. Hassan. Immunoinformatics Prediction of Peptide-Based Vaccine Against African Horse Sickness Virus. Immunome Research. 2017; 13(2): 1-14.

[29] Ahmed Hamdi Abu-haraz KAA-e, Mojahid Salah Ibrahim, Waleed Hassan Hussien, Mohammed Siddig Mohammed, Marwan Mustafa Badawi and Mohamed Ahmed Salih. Multi Epitope Peptide Vaccine Prediction against Sudan Ebola Virus Using Immuno-Informatics Approaches. Advanced Techniques in Biology & Medicine. 2017; 5(1): 1-21.

[30] Ren JCTaEC. Immunoinformatics: Current trends and future directions. Drug Discovery Today. 2009; 14.

[31] De NTaRK. Immunoinformatics: an integrated scenario. Immunology. 2010:153-68.

[32] Kumar, S., G. Stecher, and K. Tamura, *MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets.* Molecular biology and evolution, 2016. **33**(7): p. 1870-1874.

[33] Tom Hall Ib, Carlsbad, Ca. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences. 2011: 60-1.

[34] Bourne JVPaPE. Antibody-protein interactions: benchmark datasets and prediction tools evaluation. BMC Structural Biology. 2007: 1-19.

[35] Jens Erik Pontoppidan Larsen OLaMN. Improved method for predicting linear B-cell epitopes. Immunome Research. 2006: 1-7.

[36] EMILIO A. EMINI JVH, ' DEBRA S. PERLOW, AND JOSHUA BOGER. Induction of Hepatitis A Virus-Neutralizing Antibody by a Virus- Specific Synthetic Peptide. JOURNAL OF VIROLOGY,. 1985; 55: 836-9.

[37] Tongaonkar ASKaPC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS 09210. 1990; 276: 172-4.

[38] MORTEN NIELSEN CL, PEDER WORNING, SANNE LISE LAUEMØLLER, KASPER LAMBERTH, SØREN BUUS SØREN BRUNAK, AND OLE LUND. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Science. 2003:12:1007-17.

[39] Rebecca L. Tallmadge JAC, Donald C. Miller, and Douglas F. Antczak. Analysis of MHC class I genes across horse MHC haplotypes. Immunogenetics. 2010: 159-72.

[40] Claus Lundegaard OLaMN. Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers. bioinformatics. 2008; 24: 1397-8.

[41] Gabor Szalai DFA, Heinz Gerber , Sandor Lazary. Molecular cloning and characterization of horse DQB cDNA. Immunogenetics. 1994; 40: 458.

[42] Lund MNaO. NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. BMC Bioinformatics. 2009:1-10.

[43] ERIC F. PETTERSEN TDG, CONRAD C. HUANG, GREGORY S. COUCH, DANIEL M. GREENBLATT, ELAINE C. MENG, THOMAS E. FERRIN. UCSF Chimera—A Visualization System for Exploratory Research and Analysis. J Comput Chem. 2004; 25: 1605-12.

[44] Julien Maupetit PDaPT. PEP-FOLD: an online resource for de novo peptide structure prediction. Nucleic acids research. 2009; 37: 498-503.

[45] PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic acids research. 2005; 33: 363-7.

[46] Groot ASD. Immunomics: discovering new targets for vaccines and therapeutics. Drug Discovery Today. 2006; 11: 203-9.

[47] Immuno-informatics: Mining genomes for vaccine components. Immunology and Cell Biology 2002; 80: 255-69.

[48] Anne S. De Groot MA, Elizabeth M. McClaine, Leonard Moise, William D. Martin. Immunoinformatic comparison of T-cell epitopes contained in novel swine-origin influenza A (H1N1) virus with epitopes in 2008-2009 conventional influenza vaccine. Vaccine. 2009; 27 5740-7.

[49] Jiandong Shi1 JZ, Sijin Li1, Jing Sun, Yumei Teng, Meini Wu, Jianfan Li, Yanhan Li, Ningzhu Hu, Haixuan Wang, Yunzhang Hu. Epitope-Based Vaccine Target Screening against Highly Pathogenic MERS-CoV: An In Silico Approach Applied to Emerging Infectious Diseases. PloS one. 2015: 1-16.

[50] Omar Hashim Ahmed AA, Sahar Obi, Khoubieb Ali Abd_elrahman, Ahmed Hamdi and Mohammed A. Hassan. Immunoinformatic Approach for Epitope-Based Peptide Vaccine against Lagos Rabies Virus Glycoprotein G. Immunome Research. 2017: 1-8.

[51] Kenth Gustafsson LA. Structure and polymorphism of horse MHC class II DRB genes: convergent evolution in the antigen binding site. lmmunogenetics 1994; 39: 355-8.

[52] Brinkmeyer-Langford CL, Childers WJM, C. P and L. C. Skow. A conserved segmental duplication within ELA. Animal Genetics 2010: 186-95.