American Journal of Infectious Diseases and Microbiology, 2016, Vol. 4, No. 3, 61-71
Available online at http://pubs.sciepub.com/ajidm/4/3/3

DOI:10.12691/ajidm-4-3-3

Immunoinformatics Predication and in silico Modeling of Epitope-Based Peptide Vaccine Against virulent Newcastle Disease Viruses

Marwan Mustafa Badawi[1,*], Afra AbdElhamid Fadl Alla[1], Salma Sleak Alam[2], Wafa Aljack Mohamed[3], Duaa Adil Nasr-Eldin Osman[2], Samar Ali Abd Alrazig Ali[2], Entissar Mohamed Elhassan Ahmed[2], Abdah AbdElmonim Adam[4], Ranya Omar Abdullah[5], Mohamed Ahmed Salih[1]

[1]Department of Biotechnology, Africa city of Technology- Khartoum, Sudan
[2]Omdurman Islamic university- Khartoum, Sudan
[3]Ibn Sina University - Khartoum, Sudan
[4]Elrazi University - Khartoum, Sudan
[5]ALneealin University- Khartoum, Sudan
*Corresponding author: mmbadwi44new@gmail.com

Abstract Newcastle disease virus (NDV) is negative sense single stranded RNA belongs to the Avulavirus genus of the Paramyxoviridae family which can be transmitted by inhalation or ingestion. Birds infected shed these viruses in feces as well as respiratory secretions. The aim of this study is to analyze fusion (F) protein of all virulent Newcastle strains reported in NCBI database using *in silico* approaches to select all possible epitopes that can be used as a therapeutic peptide vaccine. A total of 56 virulent NDV fusion protein variants retrieved from NCBI database. The conserved regions were introduced into IEDB analysis resource to predict B and T cell epitopes, as well as predicting the binding affinity of the conserved epitopes with BF2 21:01, from the predominantly expressed chicken MHC I molecule. Epitopes with high scores in both B and T cell epitopes predicting tools were suggested. Peptide vaccine against virulent NDV is strongly supersedes the conventional vaccines, as it designed to cover variant virulent mutated strains, which will reduce the recurrent outbreaks and their huge accompanied economical loss to a minimum.

Keywords: *newcastle disease virus (NDV), peptide vaccine, Immune Epitope Database IEDB, epitopes, Vaccine*

Cite This Article: Marwan Mustafa Badawi, Afra AbdElhamid Fadl Alla, Salma Sleak Alam, Wafa Aljack Mohamed, Duaa Adil Nasr-Eldin Osman, Samar Ali Abd Alrazig Ali, Entissar Mohamed Elhassan Ahmed, Abdah AbdElmonim Adam, Ranya Omar Abdullah, and Mohamed Ahmed Salih, "Immunoinformatics Predication and in silico Modeling of Epitope-Based Peptide Vaccine Against virulent Newcastle Disease Viruses." *American Journal of Infectious Diseases and Microbiology*, vol. 4, no. 3 (2016): 61-71. doi: 10.12691/ajidm-4-3-3.

1. Introduction

Newcastle disease is viral infection affect various avian species. It was first reported from Java, Indonesia, and New- castle-upon-Tyne, England, in 1926 but there are earlier reports of similar disease outbreaks in Central Europe before this date. In early 1990 there was marked increase in reported outbreaks of ND in Western Europe, peaking with 239 outbreaks in European Union [EU] countries in 1994. In 1998 two outbreaks of virulent ND in Australia and further outbreaks were reported in 1999 and 2000. Now it is endemic in many parts of the world including countries in Asia, the Middle East, Africa, and Central and South America and considered as a great economic threat to the poultry industry [1-6].

Newcastle disease is caused by Newcastle disease virus which belongs to the *Avulavirus* genus of the *Paramyxoviridae* family which can be transmitted by inhalation or ingestion, birds infected shed these viruses in feces as well as respiratory secretions [4,7].

This enveloped virus has a negative sense, non-segmented, single stranded RNA genome of almost 15.200 nucleotides in length. It contains six genes which encode the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), large polymerase protein (L) and the two surface glycoproteins; fusion protein (F) and hemagglutinin neuraminidase (HN), [1,8,11].

There are 9 serotypes of avian paramyxovirus that is capable of infecting avian species. NDV belongs to serotype 1; the Avian Paramyxovirus type 1 (APMV-1) which can cause varying clinical signs, depending on the pathogenicity of the strain and the species of bird. NDVs have been categorized According to pathogenicity into three strains, highly virulent (velogenic) which caused severe, often fatal, illnesses in chickens, intermediate (mesogenic) that cause respiratory disease and non virulent (lentogenic) which do not usually cause disease in adult birds. [9]

The level of pathogenicity is attributed to amino acid sequence motif present in the protease cleavage site of the precursor F protein and the ability of cellular proteases to cleave the F protein. The fusion (F) protein is the most immunogenic protein of the virus and it plays important role in the beginning of infection by mediating fusion of the virus into the host cell membrane enabling viral entry into the cell membrane. It is also considered as the main target of immune response to NDV [1,8,9,10].

Vaccines are commonly used to protect chickens, pheasants, some exotic birds and other species from Newcastle disease [3], it can protect birds from clinical signs, and may decrease virus shedding and transmission. Vaccination programs for chickens usually consist of inactivated vaccines and/or live vaccines, vaccination with inactivated vaccines is time consuming, labor intensive, expensive, and often inaccurate. however, usage of NDV live vaccine is widespread but still cause disease signs, depending upon environmental conditions and the presence of complicating infections [12]. To improve the efficacy and safety of current NDV live vaccines, Scientists work on developing genetic modification of NDV DNA which is supposed to offer the same broad immunologic advantages as live vaccine without the safety concern, still the cost is a disadvantage [3,8].

Many studies showed the immunological efficacy of peptide-based vaccines against infectious diseases. The development of peptide-based vaccines has significantly advanced with the identification of specific epitopes derived from infectious pathogens. Understanding of the molecular basis of antigen recognition and HLA binding motifs has resulted in the development of designed vaccines based on motifs predicted to bind to host class I or class II MHC. [13]

The use of this type of vaccine offers practical advantages, such as inclusion of specific protective epitopes and their exposure to the immune system, exclusion of suppressive epitopes, relative ease of construction and production, chemical stability and an avoidance of any infection or autoimmune potential hazard. [13]

The aim of our study is to analyze fusion (F) protein of all virulent Newcastle strains reported in NCBI database using *in silico* approaches to select all possible epitopes that can be used as a therapeutic peptide vaccine.

2. Materials and Methods

2.1. Protein Sequence Retrieval

A total of 56 virulent Newcastle Fusion proteins variants were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein/) database in April 2016. These 56 strains retrieved were collected from different parts of the world; retrieved fusion protein strains and their accession numbers as well as date and area of collection are listed in Table 1.

2.2. Determination of Conserved Regions

The retrieved sequences were aligned to obtain conserved regions using multiple sequence alignment (MSA). Sequences aligned with the aid of ClustalW as implemented in the BioEdit program, version 7.0.9.0 (Hall, 1999) for finding the conserved regions among international virulent fusion glycoprotein variants. Later on, the candidate epitopes were analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http://www.iedb.org/), Figure 1 - Figure 3. [14,15].

Table 1. Virus Strains retrieved and their Accession numbers and area of collection

Accession Number	Date of collection	Country
NP_071469.1*	N.A	N.A
AHJ81381	2010	China
AHJ81375	2006	China
AAA46642	N.A	N.A
ACW19917	2008	China
ABQ14807	N.A	China
ACU30816	2005	Egypt
AJE26306	2013	Pakistan
AFY26252	1947	Mexico
AFY26251	1986	Dominican
AFU48618	2008	Dominican
AHZ89382	2010	China
AHV78506	1976	Argentina
AHV78505	1976	Mexico
AHV78504	1985	USA
AHV78503	2008	Belize
AHV78500	2008	Belize
AFY07432	2010	Mexico
AFK25762	2009	China
AEX01229	2008	Peru
AEA10393	2009	China
AEA10387	2009	China
AEM55586	2002	China
ADZ45536	1995	Sweden
ADG27332	1948	USA
AAU47331	N.A	Argentina
ACI62501	2008	China
ABS84266	2002	USA
ABS84264	1984	USA
ABS86968	N.A	USA
AIL25705	2008	Colombia
AIL25704	2009	Colombia
AIL25701	2009	Colombia
AIL25700	2010	Colombia
AIL25699	2010	Colombia
AIL25697	2010	Colombia
AIL25693	2009	Colombia
AIL25691	2009	Colombia
AIL25690	2009	Colombia
P10865	N.A	N.A
P12571	N.A	N.A
P12570	N.A	N.A
P35936	N.A	N.A
P12572	N.A	N.A
P14623	N.A	N.A
P06156	N.A	N.A
P33615	N.A	N.A
P33614	N.A	N.A
P33613	N.A	N.A
P33612	N.A	N.A
ACO25494	2000	China
ACO25493	2000	China
AEZ00711	1993	Netherlands
AEZ00723	2004	Ireland
AEZ00717	2004	south Africa
AEZ00705	1971	USA
AIL25694	2009	Colombia

N.A: not available.
* Reference fusion glycoprotein.

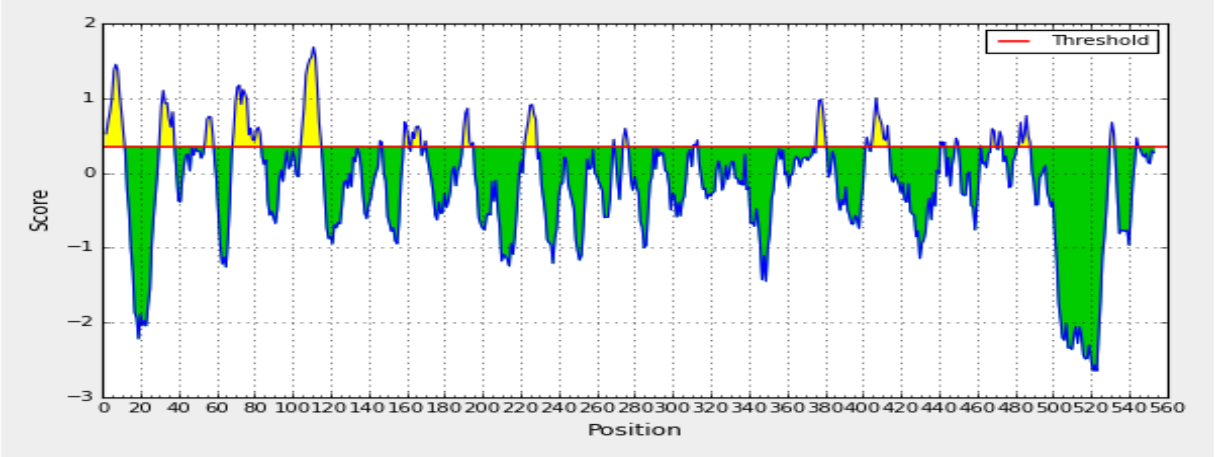

Figure 1. Bepipred Linear Epitope Prediction

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not.

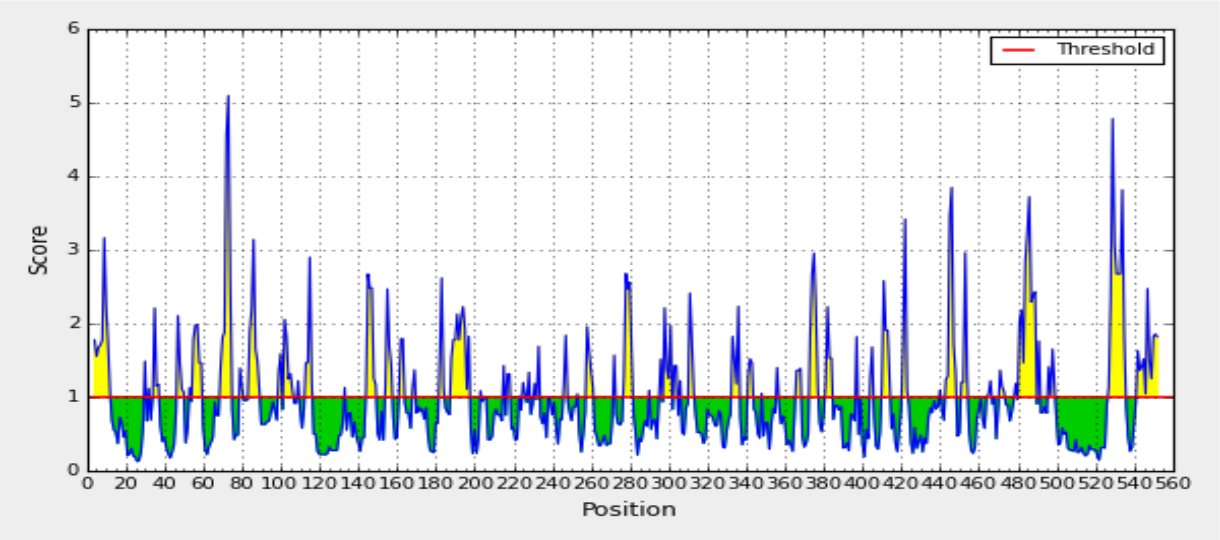

Figure 2. Emini surface accessibility prediction

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not.

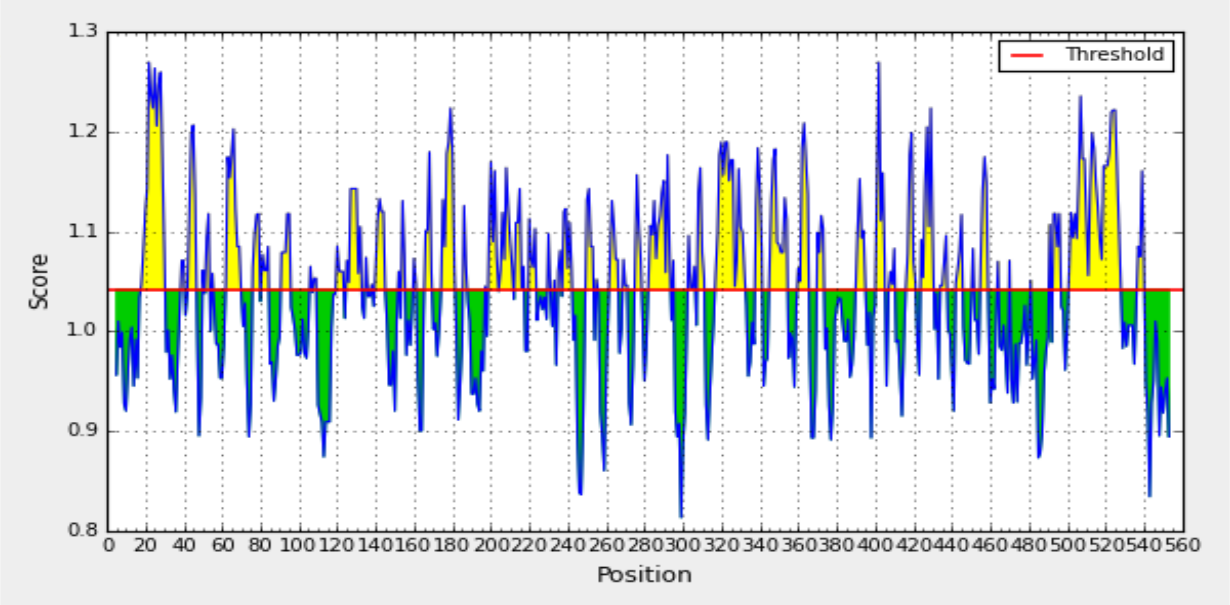

Figure 3. Kolaskar and Tongaonkar antigenicity prediction

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not.

2.3. B-cell Epitope Prediction

B cell epitope is the portion of an immunogen, which interacts with B-lymphocytes. As a result, the B-lymphocyte is differentiated into antibody-secreting plasma cell and memory cell. B cell epitope is characterized by being hydrophilic and accessible [16]. Thus, the classical propensity scale methods and hidden Markov model programmed softwares from IEDB analysis resource were used for the following aspects:

Prediction of linear B-cell Epitopes: BepiPred from immune epitope database [17] was used for linear B-cell epitopes prediction from the conserved region with a default threshold value of 0.35.

Prediction of surface accessibility: by using Emini surface accessibility prediction tool of the immune epitope data base (IEDB) ([18] the surface accessible epitopes were predicted from the conserved region holding the default threshold value 1.000 or higher.

Prediction of Epitopes antigenicity sites: [19] the kolaskar and tongaonker antigenicity method was used to determine the antigenic sites with a default threshold value of 1.042.

2.4. MHC Class I binding predictions

Analysis of peptide binding to MHC class I molecules was assessed by the IEDB MHC I prediction tool at http://tools.iedb.org/mhci/. Prediction for several organisms is supported by this tool as chicken is not among them. However, several studies suggest some similarities between HLA alleles and chicken MHC, [55,56,57,58,59] MHC-I peptide complex presentation to T lymphocytes undergo several steps, the attachment of cleaved peptides to MHC molecules step was predicted. Prediction methods can be achieved by Artificial neural network (ANN), Stabilized matrix method (SMM), or Scoring Matrices derived from Combinatorial Peptide Libraries (Comblib_Sidney2008). Consensus method was used which combines ANN, SMM and comblib methods. [20,21,22,23,24] Prior to prediction, all epitope lengths were set as 9mers, conserved epitopes that bind to many HLA alleles at score equal or less than 1.0 percentile rank were selected for further analysis.[25]

2.5. MHC Class II Binding Predictions

Analysis of peptide binding to MHC class II molecules was assessed by the IEDB MHC II prediction tool at http://tools.immuneepitope.org/mhcii/ [26,27]. For MHC-II binding predication, human allele reference set were used. MHC class II groove has the ability to bind to peptides with different lengths. This variability in binding makes prediction as difficult as less accurate [28]. There are five prediction methods for IEDB MHC II prediction tool; SMM_align, NN- align, Compinatorial Libraries, Sturniolo's method and NetMHCIIpan in addition to the consensus method. SMM-align is a matrix-based method with extensions incorporating flanking residues outside of binding grooves, NN- align uses the artificial neural networks that allows for simultaneous identification of the MHC class II binding core epitopes and binding affinity, Compinatorial Libraries apply positional scanning combinatorial libraries approach which utilizes a pool of random peptide libraries to systematically measure the contribution to MHC binding from each amino acid at each of the nine positions at the binding peptide, Sturniolo's method and NetMHCIIpan predict peptide binding to HLA-DR molecule which make them less useful. The consensus approach was used which combine the outcome of the three SMM_align, NN- align, Compinatorial Libraries methods which firstly run a random scan of Swiss-Prot proteins and achieve scores for 2,000,000 random peptides, thereafter, act as reference to rank new predictions. The consensus method uses the median rank of the three approaches as the final prediction score [29]. All conserved epitopes that bind to many alleles at score equal or less than 10-percentile rank is selected for further analysis.

2.6. Homology Modeling

Newcastle fusion protein 3D structure as well as FB2 21:01 chicken allele were obtained by phyre2, (http://www.sbg.bio.ic.ac.uk/phyre2) which uses advanced remote homology detection methods to build 3D models. Both protein sequences were retrieved from the corresponding reference sequence in NCBI. UCSF Chimera (version 1.8) was used to visualize the 3D structures, Chimera currently available within the Chimera package and available from the chimera web site (http://www.cgl.ucsf.edu/cimera). Homology modeling was achieved to establish docking, and for further verification of the service accessibility and hydrophilicity of B lymphocyte epitopes predicted, as well as to visualize all predicted T cell epitopes in the structural level [30,31].

2.6. Docking

Epitopes of MHC I alleles that predicted to bind with percentile rank below 0.5 were selected as the ligands, which are modeled using PEP- FOLD online peptide modeling tool. The receptor BF2 21:01 chicken MHC I allele 3D structure was obtained from phyre2 modeling server. Removal of water and non polar hydrogen from protein was performed using PyMOL. Autodock vina program of the pyrx python virtual screening tool was used for all dockings. Conversion of pdp files into pdpqt files were performed, as well as the default grid box features of PyRx were maintained for active site determination. PyMol was used for visualization and determination of binding affinity and polar ligands contacts and to show the suitable epitopes binding with the lowest energy. [30,32,33,34]

3. Result

3.1. B-cell Epitope Prediction

The reference F protein was analyzed using Bepipred Linear Epitope Prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity to determine the default threshold of the protein, Regarding Bepipred Linear Epitope Prediction method 7 epitopes were predicted eliciting B lymphocyte from the conserved regions (**DGR**, **AVHE**, **GPQITSP**, **PQITSP**, **IISQNYG**, **IISQNY**, **ESN**).

Emini surface accessibility prediction tool predict four of them passing the default threshold (**DGR**, **GPQITSP**, **PQITSP**, **ESN**).

Kolaskar and Tongaonkar antigenicity predict their antigenisity and only two epitopes gave score above threshold (**AVHE**, **IISQNY**), however, there is no single epitope successfully overlapped the three tools. The result is illustrated in Table 2 below and (Figure 1-Figure 3), and their positions in the structural level is shown in Figure 4.

Table 2. B-cell epitpoes prediction:

No.	Start	End	Epitope	Length	Surface accessibility [a]	Antigenicity score [b]
1	32	35	DGR	3	1.753	0.871
2	164	167	AVHE	4	0.779	1.101
3	222	228	GPQITSP	7	1.345	1.013
4	223	228	PQITSP	6	1.65	1.036
5	407	413	IISQNYG	7	0.688	1.02
6	407	412	IISQNY	6	0.845	1.045
7	483	485	ESN	3	2.021	0.88

a: default threshold value 1.000.
b: default threshold value 1.042.

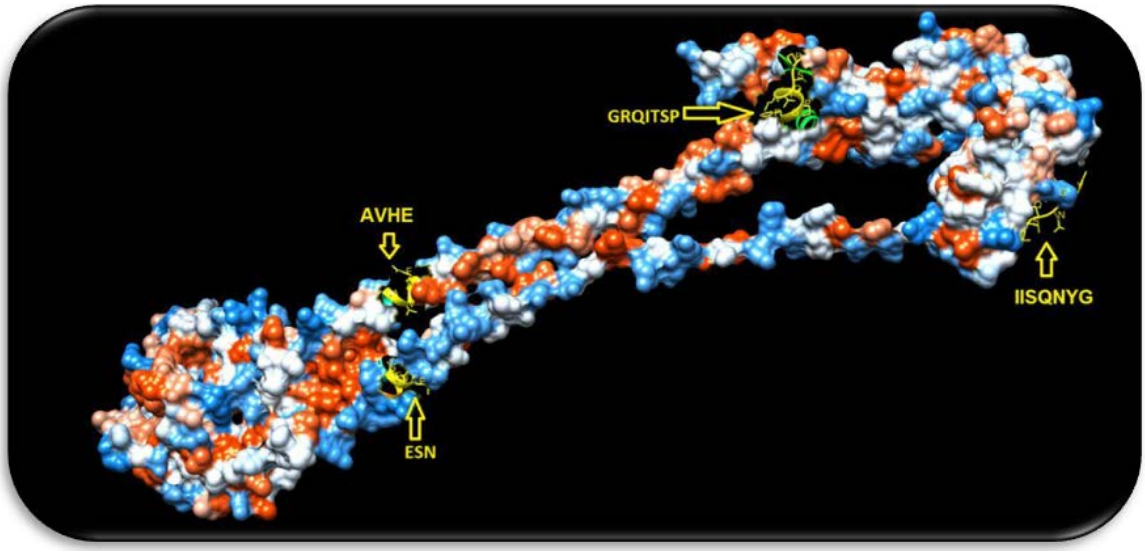

Figure 4. proposed B-Cell Epitopes in F protein

Proposed epitopes of B cell that are conserved in all virulent strains are shown in the structural level of Fusion glycoprotein of NDV.

3.2. Prediction of Cytotoxic T-lymphocyte Epitopes and Interaction with MHC Class I:

The reference F protein was analyzed using IEDB MHC-1 binding prediction tool to predict T cell epitopes suggested interacting with different types of MHC Class I alleles. Based on Consensus (ann/smm/comblib_sidney2008) with percentile rank ≤1; 6 conserved epitopes were predicted to interact with different HLA MHC-1 alleles. Epitopes and their corresponding alleles are shown in Table 3, and shown in the structural level in Figure 5.

Table 3. list of epitopes that had binding affinity with the MHC Class I alleles

Epitope	start	End	Allele	percentile Rank	SMM_ic50*
YLTELTTVF	213	221	HLA-B*46:01	0.25	608.5
			HLA-B*15:01	0.5	80.35
NYGEAVSLI	211	219	HLA-A*24:02	0.35	148
VAVGKMQQF	177	185	HLA-B*57:01	0.95	631
			HLA-B*46:01	0.4	1082
NTSACMYSK	366	374	HLA-A*68:01	0.55	36
			HLA-A*11:01	0.45	46
ISQNYGEAV	408	416	HLA-C*15:02	0.85	19.5
ASALVPKVV	315	323	HLA-C*15:02	0.95	4.5

*inhibitory concentration needed for binding, the lower is the better.

3.3. Predication of T Helper Cell Epitopes and MHC Class II Interaction Analysis

Listed below in Table 4, is the result of 15 predicted conserved epitopes found to interact with MHC-II alleles. The peptide (core), **IQALYNLAG** had high affinity to interact with seven alleles (HLA-DRB1*01:01, HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DRB1*08:02, HLA-DRB1*11:01, HLA-DRB1*15:01, HLA-DRB5*01:01), **LYLTELTTV** interact with six alleles (HLA-DPA1*01/DPB1*04:01, HLA-DPA1*01:03/DPB1*02:01, HLA DPA1*02:01/DPB1

*01:01HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*01:01 /DQB1*05:01, HLA-DRB4*01:01), while **LNLYLTELT** was found to interact with five alleles (HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DRB1*08:02, HLA-DRB1*12:01, HLA-DRB1*15:01).

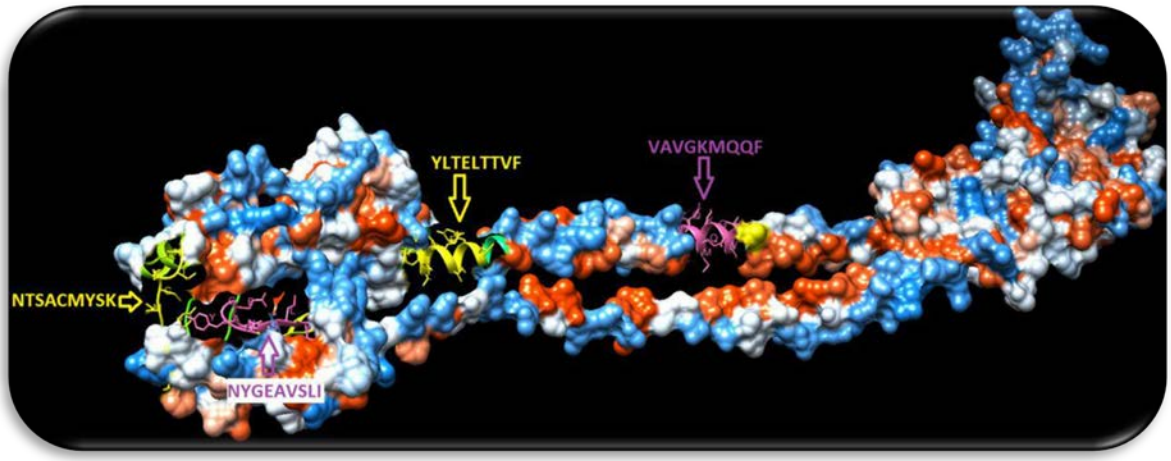

Figure 5. proposed T-Cell epitopes that interact with MHC Class I

Proposed epitopes of MHC I that are conserved in all virulent strains are shown in the structural level of Fusion glycoprotein of NDV.

Table 4. list of epitopes that had Binding affinity with the Class II alleles

Core Epitope	Allele	Percentile Rank	Peptide	Start	End	Smm ic50
FGPQITSPA	HLA-DRB1*04:01	0.83	TTVFGPQITSPALNK	218	232	97
	HLA-DRB1*04:05	8.72				607
VFGPQITSP	HLA-DRB1*04:01	0.93	TELTTVFGPQITSPA	215	219	105
LYLTELTTV	HLA-DPA1*01/DPB1*04:01	8.35	ELNLYLTELTTVFGP	209	223	820
	HLA-DPA1*01:03/DPB1*02:01	2.94				393
	HLA-DPA1*02:01/DPB1*01:01	1.31				290
	HLA-DPA1*03:01/DPB1*04:02	1.78				151
	HLA-DQA1*01:01/DQB1*05:01	7.42				3174
	HLA-DRB4*01:01	9.74				888
ISQNYGEAV	HLA-DRB1*04:05	4.17	GIISQNYGEAVSLID	406	420	273
	HLA-DRB1*13:02	1				49
YLTELTTVF	HLA-DRB1*04:01	1.14	VELNLYLTELTTVFG	208	222	122
	HLA-DRB1*08:02	6.72				1687
	HLA-DRB1*12:01	4.76				478
	HLA-DRB3*01:01	2				214
LNLYLTELT	HLA-DRB1*04:01	1.31	GVELNLYLTELTTVF	207	221	138
	HLA-DRB1*04:05	3.04				79
	HLA-DRB1*08:02	7.16				2371
	HLA-DRB1*12:01	6.39				597
	HLA-DRB1*15:01	2.6				122
IQALYNLAG	HLA-DRB1*01:01	8.87	KLTIQALYNLAGGNM	232	246	45
	HLA-DRB1*04:01	1.43				129
	HLA-DRB1*04:05	3.71				253
	HLA-DRB1*08:02	9.72				880
	HLA-DRB1*11:01	3.72				181
	HLA-DRB1*15:01	4.81				265
	HLA-DRB5*01:01	6.9				815
LTTVFGPQI	HLA-DRB1*04:05	8.44	TELTTVFGPQITSPA	215	229	586
	HLA-DRB1*09:01	2.15				232
	HLA-DRB1*11:01	9.42				2167
	HLA-DRB3*01:01	8.35				10045
LTELTTVFG	HLA-DQA1*01:02/DQB1*06:02	5.18	NLYLTELTTVFGPQI	211	225	244
	HLA-DRB1*08:02	8				864
	HLA-DRB1*09:01	5.4				258
YGEAVSLID	HLA-DQA1*03:01/DQB1*03:02	8.59	ISQNYGEAVSLIDKQ	408	422	1971
	HLA-DRB1*04:05	4.17				274
ELNLYLTEL	HLA-DQA1*01:01/DQB1*05:01	4.86	VGVELNLYLTELTTV	206	220	1280
	HLA-DQA1*05:01/DQB1*02:01	9.33				1172
ASALVPKVV	HLA-DQA1*05:01/DQB1*03:01	5.38	GFASALVPKVVTQVG	313	327	102
VGKMQQFVN	HLA-DRB1*03:01	7.19	VGKMQQFVNDQFNKT	179	193	462
	HLA-DRB3*01:01	5.8				328
NTSACMYSK	HLA-DQA1*01:02/DQB1*06:02	6.7	SCLSGNTSACMYSKT	361	375	303
ELTTVFGPQ	HLA-DPA1*01/DPB1*04:01	9.4	NLYLTELTTVFGPQI	211	225	1148

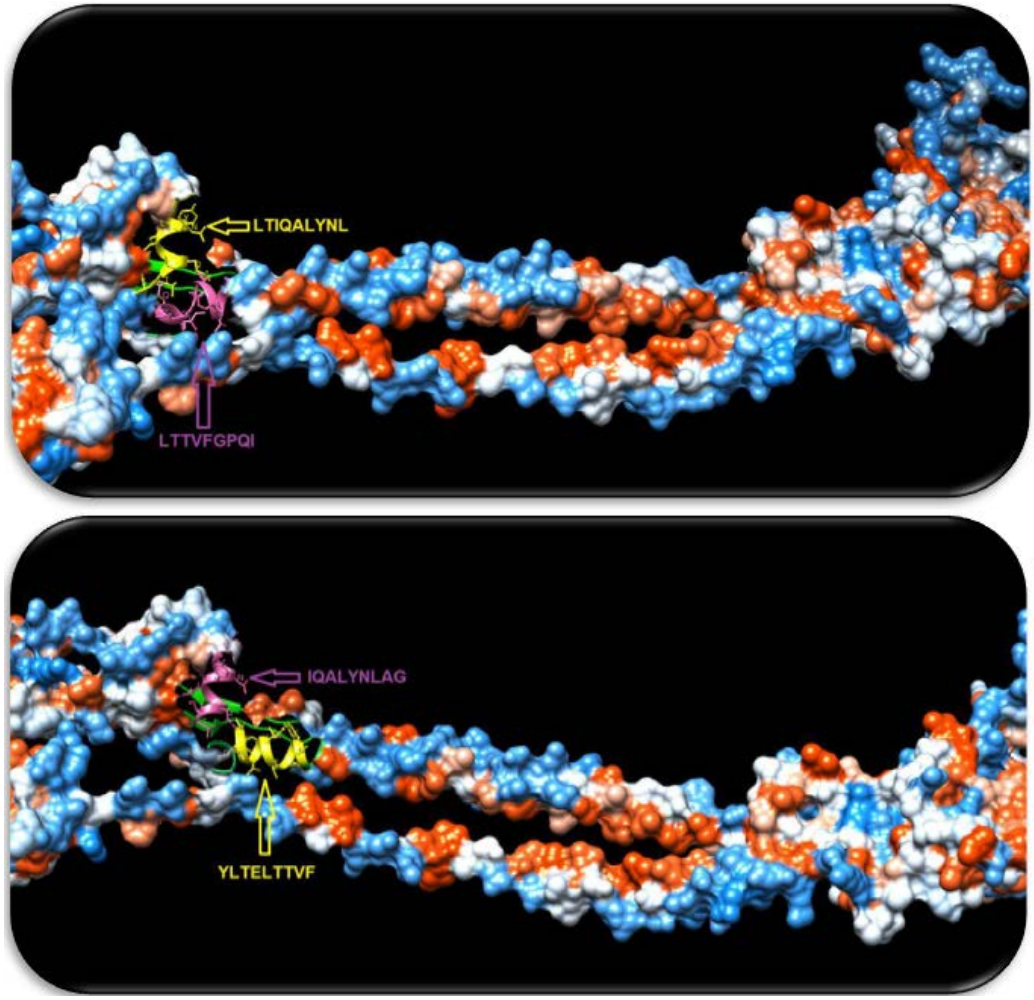

Figure 6. proposed T-Cell epitopes that interact with MHC Class II

Proposed epitopes of MHC II that are conserved in all virulent strains are shown in the structural level of fusion glycoprotein of NDV. Above and below pictures show two of proposed epitopes for each, both pictures show fusion glycoprotein of NDV, shown twice for better visualization purposes.

4. Docking

All docked epitopes appear to have good binding affinity with low binding energy, Table 5 represent the binding energy score needed for all epitopes as well as the polar contacts which were found to be around 5-10 polar bonds. epitope NYGEAVSLI is showing the best polar contacts as well as good binding energy. Figure 7 - Figure 10 visualize the polar binding interactions between receptor and epitopes in the structural level.

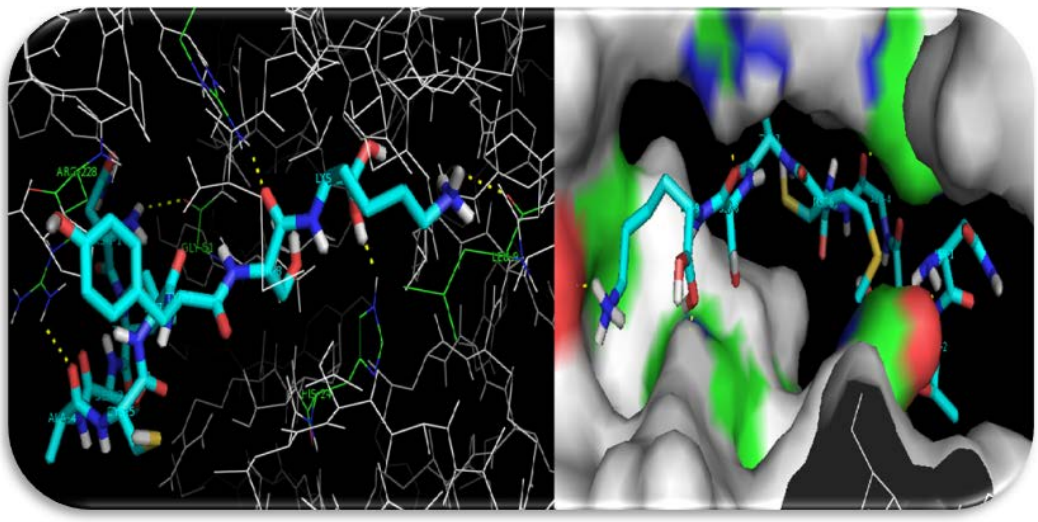

Figure 7. interaction of NTSACMYSK epitope with BF2 21:01

Left: Binding interaction of ligand shown as stick structure while the receptor shown as lines. Amino acid of receptor that binds to the ligand (epitope) are colored using default PyMol color for element. Solid dashed yellow lines represent polar contacts.
Right: Polar contacts with receptor which shown as surface by PyMol.

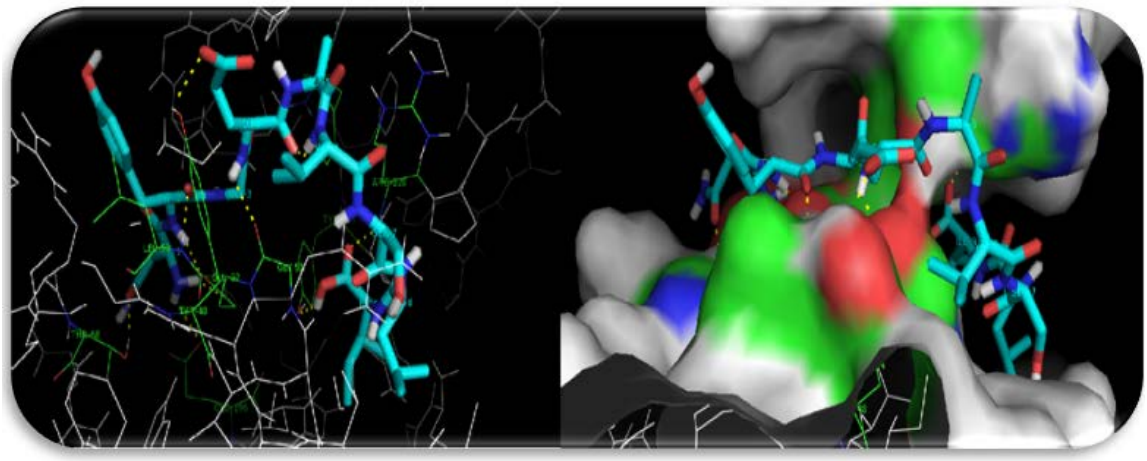

Figure 8. interaction of NYGEAVSLI epitope with BF2 21:01

Left: Binding interaction of ligand shown as stick structure while the receptor shown as lines. Amino acid of receptor that binds to the ligand (epitope) are colored using default PyMol color for element. Solid dashed yellow lines represent polar contacts. Right: Polar contacts with receptor which shown as surface by PyMol.

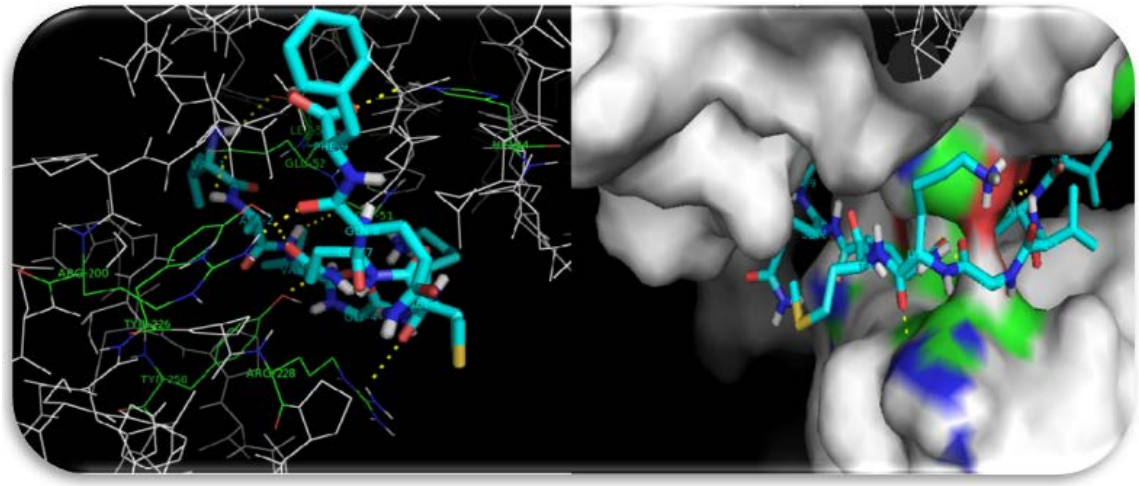

Figure 9. interaction of VAVGKMQQF epitope with BF2 21:01

Left: Binding interaction of ligand shown as stick structure while the receptor shown as lines. Amino acid of receptor that binds to the ligand (epitope) are colored using default PyMol color for element. Solid dashed yellow lines represent polar contacts. Right: Polar contacts with receptor which shown as surface by PyMol.

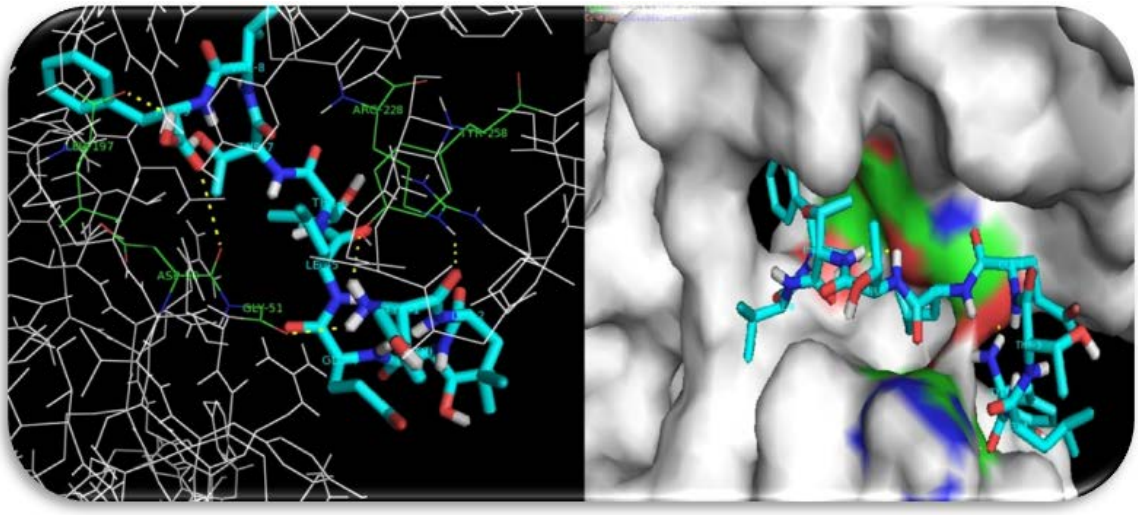

Figure 10. interaction of YLTELTTVF epitope with BF2 21:01

Left: Binding interaction of ligand shown as stick structure while the receptor shown as lines. Amino acid of receptor that binds to the ligand (epitope) are colored using default PyMol color for element. Solid dashed yellow lines represent polar contacts. Right: Polar contacts with receptor which shown as surface by PyMol

Table 5. binding affinity and polar contacts for proposed MHC I epitopes to BF2 21:01 allele:

Epitope (ligand)	binding energy (kcal/mol)[a]	Number of polar contacts[b]
NTSACMYSK	-8.4	5
NYGEAVSLI	-8.3	10
VAVGKMQQF	-7.9	8
YLTELTTVF	-7.6	5

a: energy needed for binding, the lower is the better.
b: number of polar bonds between ligand and receptor, the higher is the better.

5. Discussion

Vaccination has proven to be the mainstay in prevention of various deadly infectious diseases. Historically, live-attenuated or inactivated forms of microbial pathogens (viruses, bacteria, etc.) including NDV have been used for induction of antigen-specific responses that protect the host against infections. Based on the pathogen being used, such vaccine formulations can contain several proteins. However, protective immunity is usually dependent on a few of them, whereas the majority of proteins are unnecessary for the induction of protective immunity. Furthermore, these additional proteins may induce allergenic responses, this led to an interest of subunit vaccine, and peptide vaccine. There are many peptide vaccines under development, such as vaccine for human immunodeficiency virus (HIV), hepatitis C virus (HCV), malaria, foot and mouth disease, swine fever, influenza, anthrax, human papilloma virus (HPV), therapeutic anti-cancer vaccines for pancreatic cancer, melanoma, non-small cell lung cancer, advanced hepatocellular carcinoma, cutaneous T-cell lymphoma and B-Cell chronic lymphocytic leukemia. [35-54]

Keeping NDV under control through vaccination has proven to be difficult (Huang et al. 2003), there are several conventional live attenuated vaccines available as well as recombinant DNA vaccine for Newcastle disease viral infection; (Meulemans,1988), Fowlpox virus (Boursnell et al, 1990, Karaca et al, 1998, Olabode et al., 2010), Pigeonpox virus (Letellier et al., 1991), Herpesvirus of turkeys (Heckert et al., 1996 ; Morgan et al., 1992; Reddy et al., 1996), Marek's disease virus (Sakaguchi et al., 1998) and avian adeno-associated virus (Perozo et al., 2008), as well as other approaches include the development of subunit vaccines based on the large scale expression of NDV proteins (usually F and/or HN) using baculovirus vectors (Fukanoki et al, 2001; Lee et al, 2008; Mori et al, 1994; Nagy et al, 1991) and the use of DNA vaccines (Loke et al., 2005; Rajawat et al., 2008). The desired profile of Newcastle disease viral infection is achieved when cross-protection against variant strains, increased safety and minimal side effects are maintained, which is best addressed by designing a multi epitope conserved peptide vaccine.

Among the retrieved strains, the earliest strain (AFY26252) was collected at 1947 from Mexico, while the last strain collected among retrieved were (AJE26306) from Pakistan, as it is collected in 2013. In between are strains collected from different countries in Africa, Europe, North America and Asia as illustrated in Table 1, as a result, the conserved regions among international strains accompanied with good level of confidence.

To determine a potential and effective peptide antigen for B cell, epitopes should get above threshold scores in Bepipred linear epitope prediction, Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity prediction methods. According to Epitopes illustrated in Table 2, these epitopes are the only conserved regions from all declared virulent strains of Newcastle virus fusion glycoprotein in NCBI database until 15th of April 2016, that have high probability of activating B lymphocyte, however, these epitopes predicted satisfied Bepipred linear epitope prediction as well as Emini surface accessibility scales, but none of them succeeded the Kolaskar and Tongaonkar antigenicity prediction method. There are many antigenic sites that passed the threshold of all three predilection methods, but according to the high mutation rates in Newcastle fusion protein, none of them is observed as absolutely conserved region.

Since the immune response of T cell is long lasting response comparing with B cell, where the antigen can easily escape the antibody memory response and CD8+ T and CD4+ T cell responses play a major role in antiviral immunity, designing of vaccine against T cell epitope is much more promising. T cell activation prediction was not predicted as B cell, alleles of MHC class I & II in chicken are not as human alleles, hence there is no database or software available that can calculate the binding affinity of protein with specific chicken MHC class one and two alleles. However, there are several studies that conclude some similarities between some human and chicken MHC molecules. The conserved epitopes illustrated in (Table 3, Table 4) was found to interact with some of HLA-A, HLA-B and HLA-C alleles for MHC class I, as well as some of HLA-DR, HLA-DP and HLA-DQ alleles for MHC class II. [54,55,56,57]

Kokh M et, al, 2007 suggested a promiscuous peptide binding affinity of BF2 21:01, one from the most expressed MHC I alleles in chicken, and conclude its uniqueness in flexibility of binding different peptides. After docking this allele with the conserved epitopes of fusion glycoprotein, we found good conserved binders as shown in Table 5, those binder epitopes and specially NYGEAVSLI are the most recommended among MHC I human alleles binding epitopes. [58,59,60,61]

6. Conclusion

Conventional peptide vaccine development methods are costly, and time consuming, *in silico* prediction tools is highly appreciated as it selects specific peptides in protein, which then tested *in vitro* and *in vivo* to verify and prove the effectiveness of the proposed epitopes to induce an immune response, peptide vaccine against NDV is strongly supersedes the conventional vaccines, as it designed to cover variant virulent mutated strains, which will reduce the recurrent outbreaks and their huge accompanied economical loss to a minimum.

Acknowledgments

Authors would like to thank African City of Technology members for their cooperation.

Competing Interest

The authors declare that they have no competing interests.

References

[1] Muhammad Zubair Shabbir, Siamak Zohari, Tahir Yaqub, Jawad Nazir, Muhammad Abu Bakr Shabbir, Nadia Mukhtar, Muhammad Shafee, Muhammad Sajid, Muhammad Anees, Muhammad Abbas, Muhammad Tanveer Khan, AsadAmanat Ali, Aamir Ghafoor, Abdul Ahad, Aijaz Ali Channa, Aftab Ahmad Anjum, Nazeer Hussain, Arfan Ahmad, Mohsan Ullah Goraya, Zahid Iqbal, Sohail Ahmad Khan, Hassan bin Aslam, Kiran Zehra, Muhammad UmerSohail, Waseem Yaqub, Nisar Ahmad, Mikael Berg and Muhammad Munir. Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective. J. Virology. 2013; 10:170.

[2] Yinfeng Kang, Minsha Feng, Xiaqiong Zhao, Xu Dai, Bin Xiang, Pei Gao, Yulian Liand Tao Ren, Newcastle disease virus infection in chicken embryonic fibroblasts but not duck embryonic fibroblasts is associated with elevated host innate immune response. J. Virology. 2016; 13:41.

[3] D.J. Alexander, J.G. Bell and R.G. Alders. TECHNOLOGY REVIEW: NEWCASTLE DISEASE. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS Rome. 2004.

[4] Khorajiya, Sunanda Pandey, Priya D. Ghodasara, B. P. Joshi, K. S. Prajapati, D. J. Ghodasara and R. A. Mathakiya. Patho-epidemiological study on Genotype-XIII Newcastle disease virus infection in commercial vaccinated layer farms. Veterinary World. 2015; 8(3):372-381.

[5] B. Lomniczi, E. Wehmann, J. Herczeg, A. Ballagi-Pord´any, E. F. Kaleta, O. Werner, G. Meulemans, P. H. Jorgensen, A. P. Mant´e, A. L. J. Gielkens, I. Capua, and J. Damoser. Newcastle disease outbreaks in recent years inWestern Europe were caused by an old (VI) and a novel genotype (VII). Arch Virol. 1998; 143: 49-64.

[6] Delesa Damena Mulisa, Menbere Kidane W/Kiros, Redeat Belaineh Alemu, Melaku Sombo Keno, Alice Furaso, Alireza Heidari, Tesfaye Rufael Chibsa and Hassen Chaka Chunde. Characterization of Newcastle Disease Virus and poultry-handling practices in live poultry markets, Ethiopia. Springer Plus. 2014; 3:459.

[7] Dong-Hun Lee1, Jung-Hoon Kwon, Jin-Yong Noh, Jae-Keun Park, Seong-Su Yuk, Tseren-Ochir Erdene-Ochir, Sang-Soep Nahm, Yong-Kuk Kwon, Sang-Won Lee, Chang-Seon Song. Viscerotropicvelogenic Newcastle disease virus replication in feathers of infected chickens. J. Vet Sci. 2016; 17(1): 115-117.

[8] Masoumeh Firouzamandi1, Hassan Moeini, Davood Hosseini, Mohd Hair Bejo, Abdul Rahman Omar, ParvanehMehrbod, AiniIderis. Improved immunogenicity of Newcastle disease virus inactivated vaccine following DNA vaccination using Newcastle disease virus hemagglutinin-neuraminidase and fusion protein genes. J Vet Sci. 2016; 17(1): 21-26.

[9] AlñzCzeglédi, DorinaUjvári, EszterSomogyi, EniköWehmanna,

[10] Ortrud Werner, BélaLomniczi. Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. Virus Research 120. 2006; 36-48.

[11] K. YUSOFF, M. NESBIT, H. McCARTNEY, G. MEULEMANS, D. J. ALEXANDER, M. S. COLLINS, P. T. EMMERSON AND A. C. R. SAMSON. Location of Neutralizing Epitopes on the Fusion Protein of Newcastle Disease Virus Strain Beaudette C. J. gen. Virol. 1989; 70: 3105-3109.

[12] BEN P. H. PEETERS, OLAV S. DE LEEUW, GUUS KOCH, AND ARNO L. J. GIELKENS. Rescue of Newcastle Disease Virus from Cloned cDNA: Evidence that Cleavability of the Fusion Protein Is a Major Determinant for Virulence. J. VIROLOGY. 1999; 5001-5009.

[13] Tamar Ben-Yedidia and Ruth Arnon, Epitope-based vaccine against influenza. Expert Rev. Vaccines. 2007; 6(6).

[14] Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

[15] Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, Wheeler DK, Gabbard JL, Hix D, Sette A, Peters B. The immune epitope database (IEDB) 3.0. Nucleic Acids Res. 2014 Oct 9. pii: gku938. [Epub ahead of print] PubMed PMID: 25300482.

[16] Anayet Hasan, Mehjabeen Hossain and Md. Jibran Alam. A Computational Assay to Design an Epitope-Based Peptide Vaccine Against Saint Louis Encephalitis Virus. Bioinformatics and Biology Insights 2013:7 347-355.

[17] Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

[18] Emini EA, Hughes JV, Perlow DS, Boger J. 1985. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 55:836-839.

[19] Kolaskar AS, Tongaonkar PC. 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett276:172-174.

[20] Kim Y, Ponomarenko J, Zhu Z, Tamang D, Wang P, Greenbaum J, Lundegaard C, Sette A, Lund O, Bourne PE, Nielsen M, Peters B. 2012. Immune epitope database analysis resource. NAR.

[21] Nielsen M, Lundegaard C, Worning P, Lauemøller SL, Lamberth K, Buus S, Brunak S, Lund O. 2003. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Sci 12:1007-1017.

[22] Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, and Nielsen M. 2008. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse, and Monkey MHC class I affinities for peptides of length 8-11. NAR 36:W509-512.

[23] Peters B, Sette A. 2005. Generating quantitative models describing the sequence specificity of biological processes with the stabilized matrix method. BMC Bioinformatics 6:132.

[24] Sidney J, Assarsson E, Moore C, Ngo S, Pinilla C, Sette A, Peters B. 2008. Quantitative peptide binding motifs for 19 human and mouse MHC class I molecules derived using positional scanning combinatorial peptide libraries. Immunome Res 4:2.

[25] Kim Y, Ponomarenko J, Zhu Z, et al. Immune epitope database analysis resource. Nucleic Acids Research. 2012;40 (Web Server issue):W525-W530.

[26] Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol. 4(4):e1000048.

[27] Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M, Peters B. 2010. Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinformatics. 11:568.

[28] Pratik Narain Srivastava, Richa Jain, Shyam Dhar Dubey, Sharad Bhatnagar, Nabeel Ahmad. Prediction of Epitope-Based Peptides for Vaccine Development from Coat Proteins GP2 and VP24 of Ebola Virus Using Immunoinformatics, International Journal of Peptide Research and Therapeutics (2016) 22:119-133.

[29] Zhang, Q., Wang, P., Kim, Y., Haste-Andersen, P., Beaver, J., Bourne, P. E. et al. (2008). Immune epitope database analysis resource (IEDB-AR).Nucleic Acids Research, 36(Web Server issue), W513-W518.

[30] The Phyre2 web portal for protein modeling, prediction and analysis Kelley LA et al. Nature Protocols 10, 845-858 (2015).

[31] UCSF Chimera-a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.

[32] Shen Y, Maupetit J, Derreumaux P, Tuffery P, Improved PEP-FOLD Approach for Peptide and Miniprotein Structure Prediction, 2014.

[33] The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.

[34] L.K. Wolf, New software and Websites for the Chemical Enterprise, Chemical & Engineering News 87, 31 (2009).

[35] Bachler, B.C.; Humbert, M.; Palikuqi, B.; Siddappa, N.B.; Lakhashe, S.K.; Rasmussen, R.A.; Ruprecht, R.M. Novel biopanning strategy to identify epitopes associated with vaccine protection. J. Virol. 2013, 87, 4403-4416.

[36] Perrie, Y.; Kirby, D.; Bramwell, V.W.; Mohammed, A.R. Recent developments in particulate-based vaccines. Recent Pat. Drug Deliv. Formul. 2007, 1, 117-129.

[37] Black, M.; Trent, A.; Tirrell, M.; Olive, C. Advances in the design and delivery of peptide subunit vaccines with a focus on toll-like receptor agonists. Expert Rev. Vaccines 2010, 9, 157-173.

[38] Thompson, A.L.; Staats, H.F. Cytokines: The future of intranasal vaccine adjuvants. Clin. Dev. Immunol. 2011.

[39] Petrovsky, N.; Aguilar, J.C. Vaccine adjuvants: Current state and future trends. Immunol. Cell Biol. 2004, 82, 488-496.

[40] Sesardic, D. Synthetic peptide vaccines. J. Med. Microbiol. 1993, 39, 241-242.

[41] Liu, Y.; McNevin, J.; Zhao, H.; Tebit, D.M.; Troyer, R.M.; McSweyn, M.; Ghosh, A.K.; Shriner, D.; Arts, E.J.; McElrath, M.J.; et al. Evolution of human immunodeficiency virus type 1 cytotoxic T-lymphocyte epitopes: Fitness-balanced escape. J. Virol. 2007, 81, 12179-12188.

[42] Kolesanova, E.F.; Sanzhakov, M.A.; Kharybin, O.N. Development of the schedule for multiple parallel ―difficult Peptide synthesis on pins. Int. J. Pept. 2013.

[43] Epstein, J.E.; Giersing, B.; Mullen, G.; Moorthy, V.; Richie, T.L. Malaria vaccines: Are we getting closer? Curr. Opin. Mol. Ther. 2007, 9, 12-24.

[44] Volpina, O.M.; Gelfanov, V.M.; Yarov, A.V.; Surovoy, A.Y.; Chepurkin, A.V.; Ivanov, V.T. New virus-specific T-helper epitopes of foot-and-mouth disease viral VP1 protein. FEBS Lett. 1993, 333, 175-178.

[45] Tarradas, J.; Monso, M.; Munoz, M.; Rosell, R.; Fraile, L.; Frías, M.T.; Domingo, M.; Andreu, D.; Sobrino, F.; Ganges, L. Partial protection against classical swine fever virus elicited by dendrimeric vaccine-candidate peptides in domestic pigs. Vaccine 2011, 29, 4422-4429.

[46] Stanekova, Z.; Kiraly, J.; Stropkovska, A.; Mikušková, T.; Mucha, V.; Kostolanský, F.; Varečková, E. Heterosubtypic protective immunity against influenza a virus induced by fusion peptide of the hemagglutinin in comparison to ectodomain of M2 protein. Acta Virol. 2011, 55, 61-67.

[47] Oscherwitz, J.; Yu, F.; Cease, K.B. A synthetic peptide vaccine directed against the 2ss2–2ss3 loop of domain 2 of protective antigen protects rabbits from inhalation anthrax. J. Immunol. 2010, 185, 3661-3668.

[48] Solares, A.M.; Baladron, I.; Ramos, T.; Valenzuela, C.; Borbon, Z.; Fanjull, S.; Gonzalez, L.; Castillo, D.; Esmir, J.; Granadillo, M.; et al. Safety and immunogenicity of a human papillomavirus peptide vaccine (CIGB-228) in women with high-grade cervical intraepithelial neoplasia: first-in-human, proof-of-concept trial. ISRN Obstet. Gynecol. 2011.

[49] Bernhardt, S.L.; Gjertsen, M.K.; Trachsel, S.; Møller, M.; Eriksen, J.A.; Meo, M.; Buanes, T.; Gaudernack, G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/II study. Br. J. Cancer 2006, 95, 1474-1482.

[50] Brunsvig, P.F.; Aamdal, S.; Gjertsen, M.K.; Kvalheim, G.; Markowski-Grimsrud, C.J.; Sve, I.; Dyrhaug, M.; Trachsel, S.; Møller, M.; Eriksen, J.A.; et al. Telomerase peptide vaccination: A phase I/II study in patients with non-small cell lung cancer. Cancer Immunol. Immunother. 2006, 55, 1553-1564.

[51] Brunsvig, P.F.; Kyte, J.A.; Kersten, C.; Sundstrøm, S.; Møller, M.; Nyakas, M.; Hansen, G.L.; Gaudernack, G.; Aamdal, S. Telomerase peptide vaccination in NSCLC: A phase II trial in stage III patients vaccinated after chemoradiotherapy and an 8-year update on a phase I/II trial. Clin. Cancer Res. 2011, 17, 6847-6857.

[52] Kyte, J.A.; Gaudernack, G.; Dueland, S.; Trachsel, S.; Julsrud, L.; Aamdal, S. Telomerase peptide vaccination combined with temozolomide: A clinical trial in stage IV melanoma patients. Clin. Cancer Res. 2011, 17, 4568-4580.

[53] Greten, T.F.; Forner, A.; Korangy, F.; N'Kontchou, G.; Barget, N.; Ayuso, C.; Ormandy, L.A.; Manns, M.P.; Beaugrand, M.; Bruix, J. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. BMC Cancer 2010, 10, e209.

[54] Kyte, J.A.; Trachsel, S.; Risberg, B.; Thor, S.P.; Lislerud, K.; Gaudernack, G. Unconventional cytokine profiles and development of T cell memory in long-term survivors after cancer vaccination. Cancer Immunol. Immunother. 2009, 58, 1609-1626. 25.

[55] Spike C.A. & Lamont S.J. (1995>. - Genetic analysis of 3 loci homologous to human G9a: evidence for inkage of a class III gene with the chicken MHC. Anim. Genet., 26, 185-187.

[56] Chen, F. et al. "Character Of Chicken Polymorphic Major Histocompatibility Complex Class II Alleles Of 3 Chinese Local Breeds". Poultry Science 91.5 (2012): 1097-1104. Web.

[57] Chappell P, et al. (2015) Expression levels of MHC class I molecules are inversely correlated with promiscuity of peptide binding. eLife 4:e05345.

[58] Wallny H-J, et al. (2006) Peptide motifs of the single dominantly expressed class I molecule explain the striking MHC-determined response to Rous sarcoma virus in chickens. Proc Natl Acad Sci USA 103(5):1434-1439.

[59] Koch M, et al. (2007) Structures of an MHC class I molecule from B21 chickens illustrate promiscuous peptide binding. Immunity 27(6):885-899.

[60] Walker BA, et al. (2011) The dominantly expressed class I molecule of the chicken MHC is explained by coevolution with the polymorphic peptide transporter (TAP) genes. Proc Natl Acad Sci USA 108(20):8396-8401.

[61] Shaw I, et al. (2007) Different evolutionary histories of the two classical class I genes BF1 and BF2 illustrate drift and selection within the stable MHC haplotypes of chickens. J Immunol 178(9): 5744-5752.