Vaccine Design against Coronavirus Spike (S) Glycoprotein in Chicken: Immunoinformatic and Computational Approaches

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Research

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Abstract

Background

Infectious bronchitis (IB) is a highly contagious respiratory disease in chickens and produces economic loss within the poultry industry. It is caused by a single stranded RNA virus belonging to Coronaviridae family.

Methods

The present study used various tools in Immune Epitope Database (IEDB) to predict conserved B and T cell epitopes against IBV spike (S) protein that may perform a significant role in provoking the resistance response to IBV infection. Structural analysis, homology modelling and molecular docking were also achieved.

Results

In B cell prediction methods, three epitopes ($_{1139}KKSSYY_{1144}$, $_{1140}KSSYYT_{1145}$, $_{1141}SSYYT_{1145}$) were selected as surface, linear and antigenic epitopes based on the length and antigenicity score. Many MHCI and MHCII epitopes were predicted for IBV S protein. Among them $_{982}YYITARDMY_{990}$ and $_{983}YITARDMYM_{991}$ epitopes displayed high antigenicity, no allergenicity and no toxicity as well as great linkage with MHCI and MHCII alleles. Moreover, docking analysis of MHCI epitope produced strong binding affinity with BF₂ alleles.

Conclusion: Five conserved epitopes were expected from spike glycoprotein of IBV as the top B cell and T cell epitopes due to high antigenicity, no allergenicity and no toxicity. In addition, MHC epitopes showed great linkage with MHC alleles as well as strong interaction with BF2 alleles. These epitopes should be designed and incorporated and then tested as multi-epitope vaccine against IBV.

Backgrounds

Infectious bronchitis virus (IBV) is a single positive stranded RNA that belonging to coronavirus of the chicken (*Gallus gallus*). It is a highly contagious respiratory disease in chickens that is mainly severe for very young chicks. The signs of illness include tracheal rales, coughing, sneezing, nasal discharge and some strains may cause kidney damage [1, 2]. The disease can be transmitted by infected chickens in respiratory discharges and feces, and it can be spread by aerosol, ingestion of contaminated feed and water, and contact with contaminated equipment or clothing. The virus cannot be transmitted via eggs [3]. The disease causes economic loss within the poultry industry, affecting the performance of meat-type and egg-laying birds. The disease can affect all ages, but the clinical disease is more severe in young chicks. Chicks become more resistant to IBV-induced mortality with increasing age [4].

There are four structural proteins associated with the envelope, the spike (S), membrane (M), envelope (E), and nucleocapsid (N) protein [5]. The spike 'S' glycoprotein is located at the surface of the virion and consists of two subunits, S1 and S2.. The membrane 'M' glycoprotein is partially exposed at the surface of the virion and the nucleocapsid 'N' protein that located internally. The spike glycoprotein of IBV induces virus neutralizing (VN) and HI antibodies and has been considered as the most likely inducer of protection [2, 4]. The spike S protein is a dimer or trimer. It has two known functions; to attach the virus to receptor molecules on host cells, and to activate

fusion of the virion membrane with host cell membranes, to release the viral genome into the cell [2]. The spike gene is highly variable, especially the S1 part, due to insertions, deletions, substitutions and recombination events [6]. Application of vaccine is the most effective way of protective against pathogenic, specifically when these pathogens have a high mortality rate such as IBV and virus in general. On the other hand, the large number of serotypes and strains (genotype) of IBV make control process complicated precisely. IBV has shift and drift property [7].

Vaccination with inactivated vaccines and live-attenuated vaccines is used to control the disease. However, inactivated vaccines frequently fail to induce strong cellular immunity, while live-attenuated vaccines can contribute to the emergence of antigenic variant viruses [5]. The increasing number of new serotypes of IBV, which were caused by frequent gene mutation and recombination, are a major challenge for the prevention and control of IB disease [8].

Moreover, RNA viruses have high mutational rates, such as IBV. So the most important step in the design of cross-protective peptide vaccine against IBV is to target the conserved epitopes of different strains of IBV [5].

There is an essential need for the development of safer and more effective vaccines to control IBV. Therefore, the aim of this study is to analyze strains of spike (S) glycoprotein of infectious bronchitis virus reported in NCBI database using immunoinformatics and computational approaches to select all possible epitopes that can be used as multi-epitopes vaccine.

2. Methods

2.1. Protein Sequence Retrieval

Spike (S) protein sequences of different infectious bronchitis virus (IBV) strains were retrieved from the GeneBank of National Central Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/protein/>) database in March 2019. The sequences were saved in FASTA format (Table 1).

2.2 Structural Analysis

Reference sequence of spike S protein (NP_040831.1) was analyzed to identify the chemicals and physical properties including GRAVY (Grand average of hydropathicity), half-life, molecular weight, stability index and amino acid atomic composition using an online tool Protparam [9].

Secondary structure of spike S protein of IBV was analyzed through PSIPRED [10]. The secondary structure of protein including helix, sheet, turn, and coil parameters was predicted using GOR IV server at https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl.

TMHMM an online tool (<http://www.cbs.dtu.dk/services/TMHMM/>), used to examine the trans-membrane topology of S protein. Presence of disulphide-bonds were predicted through an online tool DIANNA v1.1. It makes prediction based on trained neural system [11]. CDD-BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) [12–14] and PFAM (<http://www.pfam.sanger.ac.uk/>) 229E [15] were used to search the defined conserved domains in the targeted protein sequences.

Table 1

Accession numbers, date and area of collection of the retrieved sequences of Spike protein sequences of IBV.

No	Accession No	Country	Year	No	Accession No	Country	Year
1	NP_040831.1*	UK	2018	47	AAV98206.1	USA	2002
2	AHX25911.1	China	2016	48	AVX27612.1	India	2004
3	AHX25902.1	China	2016	49	ALE71331.1	India	2018
4	AHX25893.1	China	2016	50	AJP16712.1	China	2015
5	AMK51938.1	China	2016	51	AJP16739.1	China	2015
6	AEP84746.1	China	2016	52	AFP50306.1	Korea	2015
7	AEP84736.1	China	2016	53	AFP50302.1	Korea	2012
8	ACX71849.1	China	2011	54	AFP50294.1	Korea	2012
9	ACX71844.1	China	2011	55	AFP50274.1	Korea	2012
10	ACX71842.1	China	2011	56	AEL12221.1	China	2012
11	AAU09490.1	China	2011	57	ADY62552.1	China	2012
12	AAV24433.1	Singapore	2005	58	ADV71785.1	Netherlands	2010
13	AAV24423.1	Singapore	2005	59	ACQ55230.1	Netherlands	2011
14	AAV21248.1	Singapore	2005	60	ARE67884.1	Pakistan	2017
15	AAV21247.1	Singapore	2005	61	ARB66180.1	China	2017
16	AAV21246.1	Singapore	2005	62	AQY55821.1	China	2017
17	AAV21245.1	Singapore	2005	63	AHX26172.1	China	2016
18	AAV21244.1	Singapore	2005	64	AHX26163.1	China	2016
19	AAV21243.1	Singapore	2005	65	AHX26154.1	China	2016
20	AAV21242.1	Singapore	2005	66	AHX26145.1	China	2016
21	AGW24533.1	India	2015	67	AHX26136.1	China	2016
22	AAW33786.1	USA	2006	68	AHX26127.1	China	2016
23	AER08740.1	Sweden	2012	69	AHX26118.1	China	2016
24	AER08739.1	Sweden	2012	70	AHX26109.1	China	2016
25	AER08729.1	Sweden	2012	71	AHX26073.1	China	2016
26	AER08728.1	Sweden	2012	72	AHX26064.1	China	2016
27	AER08727.1	Sweden	2012	73	AHX26055.1	China	2016

*Refseq

No	Accession No	Country	Year	No	Accession No	Country	Year
28	AER08726.1	Sweden	2012	74	AHX26046.1	China	2016
29	AER08725.1	Sweden	2012	75	AHX26037.1	China	2016
30	AER08724.1	Sweden	2012	76	AHX26028.1	China	2016
31	AER08723.1	Sweden	2012	77	AHX26019.1	China	2016
32	AER08722.1	Sweden	2012	78	AHX26010.1	China	2016
33	AER08721.1	Sweden	2012	79	AHX26001.1	China	2016
34	ADA83557.1	USA	2011	80	AHX25992.1	China	2016
35	ADA83467.1	USA	2011	81	AHX25983.1	China	2016
36	ABH01142.1	USA	2007	82	AHX25974.1	China	2016
37	ABH01141.1	USA	2007	83	AHX25965.1	China	2016
38	ABI26423.1	USA	2006	84	AHX25956.1	China	2016
39	AAK27168.1	China	2005	85	AHX25947.1	China	2016
40	ACH72794.1	China	2009	86	AHX25938.1	China	2016
41	AAW83034.1	China	2006	87	AHX25929.1	China	2016
42	ARS23139.1	Egypt	2014	88	ACJ50199.1	Singapore	2005
43	AHX25920.1	China	2016	89	ACO37566.1	Singapore	2005
44	ADP06504.1	USA	2012	90	AYG86360.1	SouthKorea	2018
45	AAA66578.1	UK	1995	91	AYG86347.1	SouthKorea	2018
46	AAA70235.1	USA	2002	92	AAV28722.1	China	2006
*Refseq							

2.3 Multiple Sequence Alignment And Epitope Conservancy Assessment

The retrieved sequences of IBV S protein were aligned using Clustal program and consensus sequence was generated using the multiple sequence alignment (MSA) tool, Jalview version 2.10.5.

(<http://www.jalview.org/about/jalview-scientific-advisory-committee>) [16]. Epitope conservancy analysis in Immune Epitope Database (IEDB) was used to detect potential epitope conservancy (<http://tools.iedb.org/conservancy/>) [17]. For calculating the conservancy score, the sequence identity threshold was kept at 80%.

2.4 Phylogeny Analysis

Phylogenetic tree of the retrieved sequences of spike (S) protein was performed using MEGA7.0.26 (7170509) software using maximum likelihood parameter [18].

2.5 B Cell Prediction

The Immune Epitope Database (IEDB) (<http://tools.iedb.org/mhci/>) was used to predict B and T cell epitopes of IBV reference sequence of S proteins (NP_040831.1) [19]. Linear B-cell epitopes were predicted using BepiPred from IEDB [20]. Emini surface accessibility prediction tool was used to predict epitopes located on the surface [21]. Whereas, the antigenic epitopes were investigated using kolaskar and Tongaonkar antigenicity method [22].

2.6 T-cell Epitope Prediction

The T cell epitopes were predicted in human among different alleles of major histocompatibility complex class I (MHC-I) and class II (MHCII). MHC-I binding epitopes were predicted using artificial neural networks (ANN) [23, 24]. Peptide length was set as 9 amino acids. The half maximal inhibitory concentration (IC50) values of the peptides binding to MHC-I molecule was calculated and the epitope that had IC50 with binding affinity less or equal to 300 nM were suggested as promising candidate epitopes. MHC class II molecules was performed by the IEDB MHCII prediction tool at (<http://tools.iedb.org/mhcii/>) [19]. Human MHC class II alleles (HLA DR, HLA DP and HLA DQ) were used for MHCII binding predication. NN-align method was used with IC50 less or equal to 1000 nM [25].

2.7 Antigenicity, Allergenicity And Toxicity Of Epitopes

VaxiJen v2.0 server (<http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>) was used to predict the antigenicity of the conserved regions [26]. It was used with the default prediction parameters and a threshold value of 0.4. The *in silico* allergenicity prediction of epitopes was investigated using AllerTop v .2.0 (<http://www.ddg-pharmfac.net/AllerTOP>) [27]. While ToxiPred server was used to predict the toxicity of predicted epitopes. (<http://crdd.osdd.net/raghava/toxinpred/>) [28].

2.8 Homology Modeling:

IBV reference sequences was submitted to SWISS- MODEL (<http://swiss-model.expasy.org/>) for protein structure homology modelling [29–33]. The protein sequences of BF alleles (BF2 *2101 and BF2*0401) were submitted to Raptor X server (<http://raptorx.uchicago.edu/>) to design their three D structures [34–36]. Chimera software 1.8 was used to visualize the 3D structures of the IBV reference sequences and BF alleles [37].

2.9 Molecular Docking

To perform molecular docking, 3D structure of MHC-I epitopes and 3D modeled of both BF alleles were submitted simultaneously to PatchDock online autodock tools; an automatic server for molecular docking (<https://bioinfo3d.cs.tau.ac.il/PatchDock/>) [38]. Firedock (<http://bioinfo3d.cs.tau.ac.il/FireDock/>) was used to select the five top models [38]. Visualization of the result was performed using UCSF-Chimera software 1.8 [37].

3. Results

3.1 Structural analysis

The physiochemical properties of spike S protein calculated by protparam revealed that it contained 1162 amino acids (aa) with molecular weight of 128046.70 kDa, which reflects good antigenic nature. Theoretical isoelectric point (PI) of subject protein was 7.71 which indicate its positive in nature. An isoelectric point above 7 shows positively charged protein. Approximately, 81 aa were found as negatively charged whereas 84 aa found as positively charged. Protparam computed instability-index (II) 35.53, this categories protein as stable. Aliphatic-index 86.05, which devotes a thought of proportional volume hold by aliphatic side chain and GRAVY value for protein sequence is 0.012. Half-life of protein depicted as the total time taken for its vanishing after it has been synthesized in cell, which was computed as 30 h for mammalian-reticulocytes, > 20 h for yeast, > 10 h for *Escherichia coli*. Total number of Carbon (C), Oxygen (O), Nitrogen (N), Hydrogen (H) and Sulfur (S) were entitled by Formula: $C_{5737}H_{8847}N_{1495}O_{1718}S_{56}$. Secondary structure of spike S protein of IBV was analyzed through PSIPRED and GOR IV server. The component of secondary structure prediction by GOR IV server are alpha helix (29.43%), extended strand (27.37%), beta turn (5.25%), and random coil (37.95%) (Fig. 1). DiANNA1.1 tool calculated 19 disulphides bonds (S–S) positions and assign them a score and it makes prediction based on trained neural system. The trans-membrane protein topology was checked via online tool TMHMM and it was found that residue from 1 to 1093 were exposed on the surface, while residue from 1094 to 1116 were inside trans-membrane-region and residues from 1117 to 1162 were buried within the core-region of the S protein (Fig. 1).

Two conserved domains (Corona-S1 and Corona-S2) were identified in refseq of IBV spike glycoprotein. The conserved domains were sequenced by Conserved Domain (CDD) BLAST search. It stated that corona-S1 (pfam01600) is the only member of the superfamily cl03276 and corona-S2 domain (pfam01601) is the only member of the superfamily cl20218. The top related sequences in both domains were Feline infectious peritonitis virus (strain 79-1146), Avian infectious bronchitis virus (strain Beaudette), and Human coronavirus 229E while Severe acute respiratory syndrome-related coronavirus sequences was only associated to corona-S2 domain [39].

3.2 Multiple Sequence Alignment

Jalview was used to visualize the multiple sequence alignment of the retrieved sequences. In alignment, several areas have been shown to have mutation see Fig. 2.

3.3 Phylogeny

Phylogenetic trees for targeted proteins were constricted using MEGA7.0.26 (7170509) software using maximum likelihood parameter see Fig. 3.

3.4 B-cell Epitopes

In B cell prediction methods, several epitopes using Bepipred Linear Epitope Prediction method were predicted. The conservancy percentages of these epitopes were presented in Table 2 and 3. Twenty one linear conserved

epitopes were identified after shortened of predicted epitopes. Of these, seven epitopes with different length between the positions 1139–1146 were identified as linear, surface and antigenic epitopes (see Table 3). These epitopes were $_{1139}KKSSYY_{1144}$, $_{1140}KSSYYT_{1145}$, $_{1141}SSYYTT_{1146}$, $_{1141}SSYYT_{1145}$, $_{1142}SYTT_{1146}$, $_{1142}SYTT_{1145}$, and $_{1143}YYTT_{1146}$. Three epitopes ($_{1139}KKSSYY_{1144}$, $_{1140}KSSYYT_{1145}$, $_{1141}SSYYT_{1145}$) were selected as top B cell epitopes based on the length and antigenicity score.

Table 2
Conservancy Assessment of B cell linear epitopes

Epitope no	Epitope sequence	Start	End	Epitope length	Percent of protein sequence matches at identity < = 100%
1	MTAPSSGMAW	83	92	10	89.13% (82/92)
2	GGPI	193	196	4	90.22% (83/92)
3	TGNFSD	235	240	6	97.83% (90/92)
4	GPLQGGCK	352	359	8	94.57% (87/92)
5	DSAV	450	453	4	91.30% (84/92)
6	VNPCEDV	488	494	7	96.74% (89/92)
7	RNETGSQ	512	518	7	94.57% (87/92)
8	VGQKE	642	646	5	81.52% (75/92)
9	STKPAGFNTF	656	665	10	81.52% (75/92)
10	PQNAPN	926	931	6	98.91% (91/92)
11	ANASQY	959	964	6	98.91% (91/92)
12	IVPA	966	969	4	86.96% (80/92)
13	DFDFN	1026	1030	5	84.78% (78/92)
14	SKWWNDTKHELP	1034	1045	12	94.57% (87/92)
15	GKKSSYYTT	1138	1146	9	97.83% (90/92)

Table 3
List of shortened B cell epitopes that has high score in both Emini
and kolaskar

No.	Peptide	Start	End	Length	Emini	koleskar
1	SSYYTT	1141	1146	6	2.568	1.027
2	SYTTT	1142	1146	5	2.359	1.03
3	YYTT	1143	1146	4	1.26	1.035
4	KKSSYY	1139	1144	6	4.931	1.034
5	KSSYYT	1140	1145	6	3.559	1.031
6	SSYYT	1141	1145	5	2.191	1.051
7	SYYT*	1142	1145	4	2.019	1.061

3.5 Prediction Of MHC Class I Epitopes

In this study, Human MHC class-I HLA alleles were used to explore the interaction of epitopes with MHCI alleles using epitope prediction softwares as a result of the nonexistence of chicken MHC alleles in IEDB database. MHC-1 binding prediction tool using IEDB database expected thirteen conserved epitopes of Spike protein (S) which were interacted with many alleles in cytotoxic T cell. These epitopes were *¹¹¹⁵FFMTGCCGC₁₁₂₃*, *⁵⁹⁰FNLTVTDEY₅₉₈*, *⁷³⁴GLLVLPPII₇₄₂*, *¹¹⁰⁵IFILILGW₁₁₁₃*, *¹¹³⁹KKSSYYTTF₁₁₄₇*, *¹⁰⁸⁷KTYIKWPWY₁₀₉₅*, *¹⁶⁶SVYLNGDLV₁₇₄*, *⁹⁸⁵TARDMYMPR₉₉₃*, *¹¹⁴⁵TTFDNDVVT₁₁₅₃*, *⁹⁸³YITARDMYM₉₉₁*, *¹¹⁴⁴YTTFDNDVV₁₁₅₂*, *⁹⁸²YYITARDMY₉₉₀*, *¹¹⁴³YYTTFDNDV₁₁₅₁*.

3.6 Prediction Of MHC Class II Epitopes

MHC-II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC₅₀) ≤ 1000 was used. Thirty conserved core sequences were predicted to interact with MHCII alleles. These cores were *⁶⁹⁴EDLLFTSVE₇₀₂*, *¹¹⁴⁷FDNDVVTEQ₁₁₅₅*, *¹¹¹⁵FFMTGCCGC₁₁₂₃*, *¹¹¹⁶FMTGCCGCC₁₁₂₄*, *⁵⁹⁰FNLTVTDEY₅₉₈*, *⁷³⁴GLLVLPPII₇₄₂*, *¹¹⁰⁵IFILILGW₁₁₁₃*, *⁹⁰²INECVKSQS₉₁₀*, *⁹⁸⁴ITARDMYMP₉₉₂*, *⁹⁰¹KINECVKSQ₉₀₉*, *¹¹³⁹KKSSYYTTF₁₁₄₇*, *¹¹⁴⁰KSSYYTTFD₁₁₄₈*, *¹¹⁸⁷KTYIKWPWY₁₁₉₅*, *⁷³⁵LLVLPPIIT₇₄₃*, *⁵⁹²LTVTDEYIQ₆₀₀*, *¹⁰¹⁴NKTVITTFV₁₀₂₂*, *⁸⁹³QRELATQK₉₀₁*, *⁸⁹⁴QRELATQKI₉₀₂*, *⁸⁹⁵RELATQKIN₉₀₃*, *⁵⁸⁹SFNLTVTDE₅₉₇*, *¹¹⁴¹SSYYTTFDN₁₁₄₉*, *¹⁶⁶SVYLNGDLV₁₇₄*, *¹¹⁴²SYTTFDND₁₁₅₀*, *⁹⁸⁵TARDMYMPR₉₉₃*, *¹¹⁴⁶TFDNDVVTE₁₁₅₄*, *⁵⁹³TVTDEYIQT₆₀₁*, *¹⁰¹³VNKTVITTF₁₀₂₁*, *⁹⁸³YITARDMYM₉₉₁*, *¹¹⁴⁴YTTFDNDVV₁₁₅₂*, *⁹⁸²YYITARDMY₉₉₀* and *¹¹⁴³YYTTFDNDV₁₁₅₁*.

3.7 Antigenicity, allergenicity and toxicity of MHCI and MHCII epitopes:

The predicted MHCI and MHCII epitopes were subjected to VaxiJen v2.0 server, AllerJen v2.0. and ToxiPred to predict the antigenicity, allergenicity and toxicity of predicted epitopes. Five MHCI epitopes were identified as antigenic, non-allergic and non-toxic, but only three epitopes showed high linkage with MHCI alleles which were

⁹⁸⁵TARDMYMPR₉₉₃, *⁹⁸³YITARDMYM₉₉₁* and *⁹⁸²YYITARDMY₉₉₀* (Table 6). While six MHCII epitopes were predicted as antigenic, non-allergic and non-toxic epitopes (Table 7). However, *⁹⁸³YITARDMYM₉₉₁* and *⁹⁸²YYITARDMY₉₉₀* epitopes which were also presented in MHCII prediction methods, showed high antigenicity, no allergenicity and no toxicity. These epitopes were interacted with 52 and 38 alleles in MHCII see Fig. 4.

Table 4
Antigenic, non-allergic and non-toxic MHCII epitopes

Peptide	Start	End	Antigenicity	Allele	ic50
YYITARDMY*	982	990	0.8845	HLA-A*29:02	14.52
				HLA-A*30:02	160.94
				HLA-C*14:02	27.32
YITARDMYM*	983	991	0.7901	HLA-A*02:01	233.08
				HLA-A*02:06	212.86
				HLA-C*03:03	29
				HLA-C*06:02	200.39
				HLA-C*07:01	267.22
				HLA-C*14:02	49.52
				HLA-C*15:02	77.63
TARDMYMPR*	985	993	0.6914	HLA-A*30:01	56.23
				HLA-A*31:01	14.3
				HLA-A*68:01	28.24
IIFILILGW	1105	1113	0.6749	HLA-B*57:01	78.45
				HLA-B*58:01	64.27
KKSSYYTTF	1139	1147	1.1865	HLA-A*32:01	182.52

Table 5
Antigenic, non-allergic and non-toxic MHCII epitopes

Core Sequence	Antigenicity	Peptide Sequence	Start	End	Allele	IC50
IIFILILGW	0.6914	IAFATIIFILILGWV	1100	1114	HLA-DRB1*15:01	454.6
KKSSYYTTF	0.6749	MSKCGKKSSYYTTFD	1134	1148	HLA-DPA1*01:03/DPB1*02:01	872.7
		SKCGKKSSYYTTFD	1135	1149	HLA-DPA1*01/DPB1*04:01	408.1
					HLA-DPA1*01:03/DPB1*02:01	301.5
					HLA-DPA1*02:01/DPB1*05:01	953.4
		KCGKKSSYYTTFDND	1136	1150	HLA-DPA1*01/DPB1*04:01	276.8
					HLA-DPA1*02:01/DPB1*05:01	853.9
		CGKKSSYYTTFDNDV	1137	1151	HLA-DPA1*02:01/DPB1*05:01	958.9
		MSKCGKKSSYYTTFD	1134	1148	HLA-DPA1*01:03/DPB1*02:01	872.7
KSSYYTTFD	0.6466	MSKCGKKSSYYTTFD	1134	1148	HLA-DRB1*04:05	155
		SKCGKKSSYYTTFDN	1135	1149	HLA-DRB1*04:05	125.6
		KCGKKSSYYTTFDND	1136	1150	HLA-DRB1*04:05	92.2
		CGKKSSYYTTFDNDV	1137	1151	HLA-DRB1*04:05	51.9
		GKKSSYYTTFDNDVV	1138	1152	HLA-DRB1*04:05	46.9
		KKSSYYTTFDNDVVT	1139	1153	HLA-DRB1*04:05	45.3
TARDMYMPR	0.7901	SYITARDMYMPRAI	981	995	HLA-DRB1*03:01	269.3
		YYITARDMYMPRAIT	982	996	HLA-DRB1*03:01	281.9
		YITARDMYMPRAITA	983	997	HLA-DRB1*03:01	618.8
YITARDMYM	1.1865	QVNGSYYITARDMYM	977	991	HLA-DRB1*01:01	22
					HLA-DRB1*04:01	145
					HLA-DRB1*04:04	331.2
					HLA-DRB1*07:01	20.3
					HLA-DRB3*01:01	550.7
					HLA-DRB5*01:01	227.8

Core Sequence	Antigenicity	Peptide Sequence	Start	End	Allele	IC50
		VNGSYYITARDMYMP	978	992	HLA-DQA1*01:02/DQB1*06:02	338.6
					HLA-DRB1*01:01	25.8
					HLA-DRB1*03:01	447.6
					HLA-DRB1*04:01	105.8
					HLA-DRB1*04:04	248.3
					HLA-DRB1*07:01	27.8
					HLA-DRB1*15:01	380.6
					HLA-DRB3*01:01	577.8
					HLA-DRB5*01:01	198.6
		NGSYYITARDMYMPR	979	993	HLA-DQA1*01:02/DQB1*06:02	393.3
					HLA-DQA1*05:01/DQB1*03:01	817.3
					HLA-DRB1*01:01	19.8
					HLA-DRB1*03:01	176.5
					HLA-DRB1*04:01	65.2
					HLA-DRB1*04:04	225
					HLA-DRB1*07:01	40.2
					HLA-DRB1*15:01	291.2
					HLA-DRB3*01:01	635
					HLA-DRB5*01:01	93.5
		GSYYITARDMYMPRA	980	994	HLA-DQA1*01:02/DQB1*06:02	218
					HLA-DRB1*01:01	14
					HLA-DRB1*03:01	197.3
					HLA-DRB1*04:01	47.8
					HLA-DRB1*04:04	242.4
					HLA-DRB1*07:01	57.3
					HLA-DRB1*15:01	288.6
					HLA-DRB3*01:01	780.4

Core Sequence	Antigenicity	Peptide Sequence	Start	End	Allele	IC50
		SYYITARDMYMPRAI	981	995	HLA-DRB5*01:01	61.4
					HLA-DRB1*01:01	23.1
					HLA-DRB1*04:01	65.3
					HLA-DRB1*04:04	249.2
					HLA-DRB1*04:05	356.4
					HLA-DRB1*07:01	72.2
					HLA-DRB1*15:01	284.7
					HLA-DRB5*01:01	87.4
		YYITARDMYMPRAIT	982	996	HLA-DRB1*01:01	40.8
					HLA-DRB1*04:01	108.8
					HLA-DRB1*04:04	269.1
					HLA-DRB1*04:05	706.3
					HLA-DRB1*07:01	160.6
					HLA-DRB5*01:01	121.3
		YITARDMYMPRAITA	983	997	HLA-DRB1*04:01	145.4
					HLA-DRB1*04:04	652.4
					HLA-DRB1*07:01	355.4
					HLA-DRB1*08:02	955
					HLA-DRB5*01:01	206.9
YYITARDMY	0.8845	IQVNGSYYITARDMY	976	990	HLA-DQA1*05:01/DQB1*02:01	491.6
					HLA-DRB1*04:01	723.4
					HLA-DRB1*04:04	819.7
					HLA-DRB1*11:01	72
					HLA-DRB1*11:01	72
		QVNGSYYITARDMYM	977	991	HLA-DPA1*01/DPB1*04:01	710.8
					HLA-DPA1*01:03/DPB1*02:01	875.8
					HLA-DQA1*05:01/DQB1*02:01	292.7

Core Sequence	Antigenicity	Peptide Sequence	Start	End	Allele	IC50
					HLA-DRB1*03:01	588
					HLA-DRB1*11:01	32.4
					HLA-DRB1*11:01	32.4
					HLA-DPA1*01/DPB1*04:01	557.6
					HLA-DPA1*01:03/DPB1*02:01	860.8
					HLA-DQA1*05:01/DQB1*02:01	311.9
					HLA-DRB1*11:01	17.9
					HLA-DRB1*11:01	17.9
		NGSYYITARDMYMPR	979	993	HLA-DPA1*01/DPB1*04:01	503
					HLA-DPA1*01:03/DPB1*02:01	763.2
					HLA-DQA1*05:01/DQB1*02:01	387.6
					HLA-DRB1*09:01	858.7
					HLA-DRB1*09:01	858.7
					HLA-DRB1*11:01	11
					HLA-DRB1*11:01	11
		GSYYITARDMYMPRA	980	994	HLA-DPA1*01/DPB1*04:01	504.5
					HLA-DPA1*01:03/DPB1*02:01	790.4
					HLA-DQA1*05:01/DQB1*02:01	482.9
					HLA-DRB1*11:01	15.2
					HLA-DRB1*11:01	15.2
		SYYITARDMYMPRAI	981	995	HLA-DPA1*01/DPB1*04:01	480
					HLA-DPA1*01:03/DPB1*02:01	733.8
					HLA-DQA1*05:01/DQB1*02:01	526.3

Core Sequence	Antigenicity	Peptide Sequence	Start	End	Allele	IC50
		YYITARDMYMPRAIT	982	996	HLA-DRB1*11:01	26.8
					HLA-DRB1*11:01	26.8
					HLA-DPA1*01/DPB1*04:01	705.6
					HLA-DPA1*01:03/DPB1*02:01	931.5
					HLA-DQA1*05:01/DQB1*02:01	678.7
					HLA-DRB1*11:01	51.8
					HLA-DRB1*11:01	51.8

3.8 Molecular Docking

Molecular docking was achieved using peptide-binding groove affinity by docking MHCI alleles with chicken BF alleles (BF2*2101 & BF2*0401). The chicken alleles were used as receptors, and the top MHCI epitopes *⁹⁸²YYITARDMY₉₉₀*, *⁹⁸³YITARDMYM₉₉₁* and *⁹⁸⁵TARDMYMPR₉₉₃* were used as ligands. Docking of *⁹⁸³YITARDMYM₉₉₁* epitope with BF2*2101 and BF₂*0401 alleles showed – 72.11 and – 37.39 global energy respectively which indicates the strong binding affinity between the ligands and the receptors compared to other epitopes (Figs. 5, 6 and 7). In general, the global binding affinity of ligands with the receptor BF2*2101 alleles was found to be lower compared to BF2*0401, which indicates strong interaction between the receptor and ligands.

Discussion:

Acquired immunity results in the activation of antigen-specific effector mechanisms including B-cells (humoral), T-cells (cellular), macrophages, and the production of memory cells [4]. The use of B cells and T cells epitopes as a means to induce cellular and humoral immunity is likely to lead to broad based vaccines that could reduce the challenges in using of conventional attenuated vaccine [40]. There are several potential advantages offered by peptide vaccine over traditional organism vaccines. Most importantly, it allows the immune response to focus only on relevant epitopes and avoid those that lead to non-protective responses, immune evasion, or unwanted side effects, such as autoimmunity [41].

Vaccination studies with IBV have always focused on humoral immune responses in relation to protection [4]. Acquired immunity results in the activation of antigen-specific effector mechanisms including B-cells (humoral), T-cells (cellular) and macrophages, and the production of memory cells [4]. Chickens develop a good humoral response to IBV infections, which measured by ELISA, virus neutralizing (VN) and haemagglutination-inhibition HI antibodies tests [42]. It is known that S1 glycoprotein of IBV responsible of virus neutralizing (VN) and haemagglutination-inhibition HI antibodies and has been considered as the most likely inducer of protection [4].

Recently multi peptide vaccines using immunoinformatics tools was performed in Sudan for several viral diseases in chicken such as ILTV, fowlpox, Newcastle and marek's disease virus [43–46].

In this study, the physiochemical properties of spike S protein were computed using protparam. The protein reflects good stability and antigenic nature. The secondary structure prediction by GOR IV server revealed that the protein contained alpha helix (29.43%), extended strand (27.37%), beta turn (5.25%), and random coil (37.95%). DiANNA1.1 tool calculated 19 disulphides bonds (S–S) positions and the trans-membrane protein topology using TMHMM tool revealed that residue from 1 to 1093 were exposed on the surface, while residue from 1094 to 1116 were inside trans-membrane-region and residues from 1117 to 1162 were buried within the core-region of the S protein. In addition, Corona-S1 and Corona S2 domains were identified in refseq of IBV spike glycoprotein using Conserved Domain (CDD) BLAST search. The top associated sequences in both domains were Feline infectious peritonitis virus (strain 79-1146), Avian infectious bronchitis virus (strain Beaudette), and Human coronavirus 229E whereas Sever acute respiratory syndrome- related coronavirus sequences was only related to corona-S2 domain.

In B cell methods, seven shortened conserved epitopes ($_{1139}KKSSYY_{1144}$, $_{1140}KSSYYT_{1145}$, $_{1141}SSYYTT_{1146}$, $_{1141}SSYYT_{1145}$, $_{1142}SYYYTT_{1146}$, $_{1142}SYYT_{1145}$, and $_{1143}YYTT_{1146}$) were predicted from B cell prediction methods as surface, linear and antigenic epitopes. These epitopes were adjacent to each other from the position 1139–1146. This result is consistent with the results of conventional vaccines studies. [42]. In a similar study, using BepiPred epitope prediction server version 1, only one epitope (YTSNETTDVTS^{175–185}) was predicted within the S1 glycoprotein of M41 IBV strains and three such epitopes (VSNASPNSGGVD^{279–290}, HPKCNFRPENI^{328–338}, NETNNAGSVSDCTAGT^{54–69}) were predicted in CR88 IBV strains [40]. Linear B cell epitopes have been reported to play a role in virus neutralization [40]. IEDB prediction tool was used to predict linear, surface and antigenic epitopes based on the properties of amino acids such as hydrophilicity, surface accessibility, flexibility, and antigenicity [43].

Cytotoxic T lymphocytes (CTL) provide a critical arm of the immune system in eliminating autologous cells expressing foreign antigen. Unlike humoral immunity, the specificity of CTL activation depends on membrane receptors rather than secreted molecules, and antigen receptors of CTL interact with peptide determinants only in association with matched major histocompatibility complex (MHC) molecules. Virus-specific CTL have been shown to be important, if not critical, for resolution of infection and elimination of viral shedding [1]. It is stated that, the major histocompatibility complex MHC restricted CTL response can be associated with decreases in viral load, CD8⁺ lymphocytes were mostly responsible for the observed protection [1, 47]. Cytotoxic T-lymphocyte (CTL) responses to infectious bronchitis virus (IBV) were determined at regular intervals between 3 and 30 days post infection [1].

However, MHCI prediction methods showed three conserved CTL epitopes $_{985}TARDMYMPR_{993}$, $_{983}YITARDMYM_{991}$ and $_{982}YYITARDMY_{990}$ as they linkage with 7 and 3 human MHCI alleles respectively and showed high antigenicity, no allergenicity and no toxicity. Recent studies showed that vigorous cytotoxic T lymphocyte (CTL) responses that correlate with initial decrease in infection and illness can be detected after IBV infection [47]. It was established that the CD8⁺ T cells were exhausted without CD4⁺ helper T cells. CD4⁺ T cells do not appear to be important in initially resolving IBV infection in chickens [47].

In MHCII prediction method, several core peptides were predicted to interact with MHCII alleles, but surprisingly the top core peptides were also *₉₈₃YITARDMYM₉₉₁* and *₉₈₂YYITARDMY₉₉₀* which were presented in MHCI prediction methods. They linked with 52 and 38 human alleles respectively. These epitopes were showed high antigenicity, no allergincity and no toxicity. Moreover, the physiochemical properties of spike protein were also analyzed confirmed that protein has appositively charge and stable.

Molecular docking was performed to display the interaction between BF alleles (BF2*2101 & BF2*0401) and predicting MHCI epitopes (*₉₈₂YYITARDMY₉₉₀*, *₉₈₃YITARDMYM₉₉₁* and *₉₈₅TARDMYMPR₉₉₃*). The 3D structures of MHC class I binding peptides were designed using PEPFOLD and docked with BF alleles via Patchdock server. Docking of *₉₈₃YITARDMYM₉₉₁* epitope with both BF2 alleles produced strong binding affinity (– 72.11 and – 37.97 global energy respectively) followed by *₉₈₂YYITARDMY₉₉₀* (– 64.68 and – 37.57 global energy respectively). This indicates the strong interaction between the ligand and the receptor compared to other epitopes (see Figs. 5, 6 and 7). The interaction of ligands with the receptor BF2*2101 alleles was stronger compared to BF2*0401. The predicted epitopes should be tested for therapeutic potency in future studies to prove their safety and efficacy.

Conclusion:

Peptide vaccine was found to be an effective and powerful approach to a variety of pathogens. Peptides may have the potential to act as safe, non-infective, well-specific, stable vaccines. Peptide-based vaccine can correspond to highly conserved regions required for the pathogen's function and can elicit both humoral and cellular immune responses.

In this study, five epitopes were predicted from spike glycoprotein of IBV as the best B cell (*₁₁₃₉KKSSYY₁₁₄₄*, *₁₁₄₀KSSYYT₁₁₄₅* and *₁₁₄₁SSYYT₁₁₄₅*) and T cell epitopes (*₉₈₂YYITARDMY₉₉₀* and *₉₈₃YITARDMYM₉₉₁*) due to high antigenicity, no allergenicity and no toxicity as well as great linkage of MHC epitopes with MHCI MHCII alleles. These epitopes should be designed and incorporated and then tested as multi-epitope vaccine against IBV and may act as a potential peptide vaccine to control IBV infection in chicken by inducing humoral and cellular responses.

Peptide vaccine against IBV spike protein (S) is strongly supersedes the conventional vaccines, as it designed to cover all strains in different serotypes, which might be reduce the frequent outbreaks and their huge accompanied economical loss to a minimum.

Abbreviations

IB: Infectious bronchitis; IBV: Infectious Bronchitis Virus; IEDB: Immune Epitope Database; S: spike; MHC: major histocompatibility complex; BF: The genetic polymorphism of properdin factor B; refseq: reference sequence; NCBI: National Central Biotechnology Information; MSA: multiple sequence alignment; GRAVY; Grand average of hydropathicity; CDD: Conserved Domain Database; IC50: The half maximal inhibitory concentration; ANN: artificial neural networks; NN-align: artificial neural network-based alignment; HLA: The human leukocyte antigen; CTL: Cytotoxic T lymphocytes.

Declarations

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Authors' contributions

Eman, A. Awadelkareem and Sumaia A. Ali designed this study, accomplished the experiments and analyze the results. Sumaia A. Ali interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Seo SH, Collisson EW. Specific cytotoxic T lymphocytes are involved in in vivo clearance of infectious bronchitis virus. *Journal of virology*. 1997;71:5173–7.
2. Cavanagh D. Coronavirus avian infectious bronchitis virus. *Veterinary research*. 2007;38:281–97.
3. Ignjatovic J, Sapats S. Avian infectious bronchitis virus. *Revue Scientifique et Technique-Office International des Epizooties*. 2000;19:493–501.
4. Raj GD, Jones R. Infectious bronchitis virus: immunopathogenesis of infection in the chicken. *Avian Pathol*. 1997;26:677–706.
5. Yang T, Wang H-N, Wang X, Tang J-N, Lu D, Zhang Y-F, et al. The protective immune response against infectious bronchitis virus induced by multi-epitope based peptide vaccines. *Bioscience, biotechnology, and biochemistry*. 2009:0906031485-.

6. Abro SH, Ullman K, Belák S, Baule C. Bioinformatics and evolutionary insight on the spike glycoprotein gene of QX-like and Massachusetts strains of infectious bronchitis virus. *Virol J.* 2012;9:211.
7. Abdel-Moneim A, Madbouly H, Gelb J, Ladman B. Isolation and identification of Egypt/Beni-Seuf/01 a novel genotype of infectious bronchitis virus. *VETERINARY MEDICAL JOURNAL-GIZA-*. 2002;50:1065-78.
8. Mo M-L, Hong S-M, Kwon H-J, Kim I-H, Song C-S, Kim J-H. Genetic diversity of spike, 3a, 3b and e genes of infectious bronchitis viruses and emergence of new recombinants in Korea. *Viruses.* 2013;5:550–67.
9. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 2003;31:3784–8.
10. Buchan DW, Minneci F, Nugent TC, Bryson K, Jones DT. Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Res.* 2013;41:W349–57.
11. Ferrè F, Clote P. DiANNA 11: an extension of the DiANNA web server for ternary cysteine classification. *Nucleic acids research.* 2006;34:W182–5.23.
12. Altschul SF1MT, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 1997;25:3389–402.
13. Marchler-Bauer A, AJ, Derbyshire MK, DeWeese-Scott C, Gonzales NR, Gwadz M, Hao L, He S, Hurwitz DI, Jackson JD, Ke Z, Krylov D, Lanczycki CJ, Liebert CA, Liu C, Lu F, Lu S, Marchler GH, Mullokandov M, Song JS, Thanki N, Yamashita RA, Yin JJ, Zhang D, Bryant SH. CDD: a conserved domain database for interactive domain family analysis. *Nucleic Acids Res.* 2007;35:(Database issue):D237-40. Epub 2006 Nov 29.
14. SR1. E. Profile hidden Markov models.. *Bioinformatics* 1998;14:755–63.
15. Bateman ABE, Cerruti L, Durbin R, Etwiller L, Eddy SR, Griffiths-Jones S, Howe KL, Marshall M, Sonnhammer EL. The Pfam protein families database. *Nucleic Acids Res.* 2002;30:276–80.
16. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics.* 2009;25:1189–91.
17. Bui H-H, Sidney J, Li W, Fusseder N, Sette A. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. *BMC Bioinform.* 2007;8:361.
18. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology evolution.* 2016;33:1870–4.
19. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, et al. The immune epitope database (IEDB) 3.0. *Nucleic acids research.* 2014;43:D405-D12.
20. Larsen JE, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. *Immunome research.* 2006;2:2.
21. Emini EA, Hughes JV, Perlow D, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *Journal of virology.* 1985;55:836–9.
22. Kolaskar A, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett.* 1990;276:172–4.
23. Patronov A, Doytchinova I. T-cell epitope vaccine design by immunoinformatics. *Open biology.* 2013;3:120139.
24. Abdelbagi M, Hassan T, Shihabeldin M, Bashir S, Ahmed E. Immunoinformatics Prediction of Peptide-Based Vaccine Against African Horse Sickness Virus. *Immunome Res.* 2017;13:2.

25. Nielsen M, Lund O. NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. *BMC Bioinform.* 2009;10:296.
26. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinform.* 2007;8:4.
27. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v. 2—a server for in silico prediction of allergens. *J Mol Model.* 2014;20:2278.
28. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GP, et al. In silico approach for predicting toxicity of peptides and proteins. *PloS one.* 2013;8.
29. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018;46:W296–303.
30. Bienert S, Waterhouse A, de Beer TAP, Tauriello G, Studer G, Bordoli L, Schwede T. The SWISS-MODEL Repository - new features and functionality. *Nucleic Acids Res.* 2017;45:D313-D9.
31. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. *Electrophoresis.* 2009;30:162-S73.
32. Benkert P, Biasini M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics.* 2011;27:343–50.
33. Bertoni M, Kiefer F, Biasini M, Bordoli L, Schwede T. Modeling protein quaternary structure of homo- and hetero-oligomers beyond binary interactions by homology. *Scientific Reports.* 2017;7.
34. Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, et al. Template-based protein structure modeling using the RaptorX web server. *Nature protocols.* 2012;7:1511.
35. Peng J, Xu J. RaptorX: exploiting structure information for protein alignment by statistical inference. *Proteins: Struct Funct Bioinf.* 2011;79:161–71.
36. Peng J, Xu J. A multiple-template approach to protein threading. *Proteins: Struct Funct Bioinf.* 2011;79:1930–9.
37. Chan WM, Rogers SE, Nash SM, Buning PG, Meakin R. User's manual for Chimera grid tools, version 1.8. NASA Ames Research Center, URL: <http://people.nas.nasa.gov/~rogers/cgt/doc/man.html> [cited 19 July 2006]. 2003.
38. Andrusier N, Nussinov R, Wolfson HJ. FireDock: fast interaction refinement in molecular docking. *Proteins: Struct Funct Bioinf.* 2007;69:139–59.
39. Gen J. Cloning and sequencing of the gene encoding the spike protein of the coronavirus IBV. *Viol.* 1985;66:719–26.
40. Bande F, Arshad SS, Hair Bejo M, Kadkhodaei S, Omar AR. Prediction and in silico identification of novel B-cells and T-cells epitopes in the S1-spike glycoprotein of M41 and CR88 (793/B) infectious bronchitis virus serotypes for application in peptide vaccines. *Advances in bioinformatics.* 2016; 2016.
41. Reche PA, Fernandez-Caldas E, Flower DR, Fridkis-Hareli M, Hoshino Y. Peptide-based immunotherapeutics and vaccines. *Journal of immunology research.* 2014;2014.
42. Sylvester SA, Dhama K, Kataria J, Rahul S, Mahendran M. Avian infectious bronchitis: A review. *Indian Journal of Comparative Microbiology Immunology infectious Diseases.* 2005;26:1–14.

43. Ali SA, Almofti YA, Abd-elrahman KA. Immunoinformatics Approach for Multiepitopes Vaccine Prediction against Glycoprotein B of Avian Infectious Laryngotracheitis Virus. *Advances in Bioinformatics*. 2019;2019.

44. Idris S, Salih S, Basheir M, Elhadi A, Kamel S, Abd-elrahman K, et al. In silico Prediction of Peptide based Vaccine against Fowlpox Virus (FPV). *Immunome Research*. 2018;14:1–11.

45. Badawi MM, Fadl Alla A, Alam SS, Mohamed WA, Osman D, Alrazig Ali S, et al. Immunoinformatics prediction and in silico modeling of epitope-based peptide vaccine against virulent Newcastle disease viruses. *Am J Infectious Dis Microbiol*. 2016;4:61–71.

46. Bashir S, Abd-elrahman KA, Hassan MA, Almofti YA. Multi Epitope Based Peptide Vaccine against Marek's Disease Virus Serotype 1 Glycoprotein H and B. *American Journal of Microbiological Research*. 2018;6:124–39.

47. Pei J, Briles WE, Collisson EW. Memory T cells protect chicks from acute infectious bronchitis virus infection. *Virology*. 2003;306:376–84.

Figures

Figure 1

(a): The secondary structure of IBV spike protein; (b) transmembranr topology of spike protein; (c) the position of disulphides bond (S–S) in spike protein of IBV.

Figure 2

Multiple sequence alignment of spike (S) protein of IBV visualized by Jalview 2.10.5. Yellow color bar and star sign indicate the full conservation. The brown region indicates the mismatched sequences among them. Black bars show the consensus logo sequence and yellow color indicates good quality.

Figure 3

Phylogenetic tree of retrieved strains of Spike protein using MEGA7.0.26 software.

Figure 4

(a): The 3 D structure of Spike (S) glycoprotein of IBV using chimera picturing tool. (b) The position of proposed MHC I and MHC II epitopes of IBV (green colour) illustrated by UCSF-Chimera visualization tool.

Peptide	Receptor	Energy	Attractive vdw
YITARDMYM	BF ₂ 2101	- 72.11	- 37.79
	BF ₂ 0401	- 30.33	- 38.52

Figure 5

Docking of YITARDMYM with BF2 alleles

Peptide	Receptor	Energy	Attractive vdw
YYITARDMY	BF ₂ 2101	- 64.68	- 35.26
	BF ₂ 0401	- 37.57	- 23.93

Figure 6

Docking of YYITARDMY with BF2 alleles

Peptide	Receptor	Energy	Attractive vdw
TARDMYMPR	BF ₂ 2101	- 45.61	-30.25
	BF ₂ 0401	- 35.47	-28.81

Figure 7