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EPITOPE VACCINE DESIGN FOR VARIANT 2 STRAINS CIRCULATING IN IRAQ AND THE MIDDLE EAST

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ABSTRACT: Infectious bronchitis virus causes infectious bronchitis in chickens. Variant 2 is a virulent strain circulating in the Middle East, Iraq is one of them, which harbor the strain at high rate causes a severe economic loos. Sequences of variable S1 glycoprotein were collected, consensus sequence derived and used in computational epitope vaccine design. The results gave one B cell epitope "DFMYGSYHPKCDFRPETIN" with very high antigenic score. T cell (CTL) epitope prediction produced four epitopes "QTQTAQSGY, FNFSFLSSF, FSFLSSFVY, NSLSVSLAY" with desirable criteria and react with many MHC I alleles, and docked with BF2*21:01 and BF2.0401 chicken MHC I molecules. T helper cell epitopes prediction gave eight epitopes "GYYNFNFSFLSSFVY, KFIVYRETSVNTTLV, LTNFTFTNVSNALPN, TG GVNTINIYQTQTA, TINIYQTQTAQSGYY, YNFNFSFLSSFVYKQ, NNGL WFNSLSVSLAY, NGLWFNSLSVSLAYG" reacting with many MHC II alleles. These epitopes can be used for preparation of vaccine to be investigated in wet lab experiments.

INTRODUCTION: Infectious bronchitis is contiguous disease caused by Infectious Bronchitis Virus (IBV). *Gallus gallus* family and Pheasants (*Phasianus spp*) considered as natural hosts for the IBV. It is evident that IBV has become endemic worldwide [1, 2]. There is no unifying nomenclature for IBV genotypes, and the names based primarily on geographic areas, but recently a method to define IBV strains was but forward depending on complete sequence of (S1) spike glycoprotein gene [3, 4]. The virus belongs to Corona virus group with high changeable feature due to many reasons, among them it's large genome and replication strategy.

QUICK RESPONSE CODE

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Viral genetic mutations and recombination events give rise to district IBV genotypes, serotypes and pathotypes [1, 3, 5], the high frequency of appearing new IBV variants is distinguished characteristic of this virus among other Corona viruses [6]. The virus has many structural proteins include spike glycoprotein; S1 plays a major role in viral attachment, diversity and antibody neutralization.

Mutations and recombination take place in both structural and non-structural proteins, and most of them have been detected in S1 gene, this variation about 20-25% and could be up to 50%, this affects the cross-protection toward virus strains [1, 3], the variations in S1 glycoprotein are used in determination of new viral genotypes and possibly the antiviral response [1, 4]. S1 subunit located outside the virus contains about three hypervariable regions (HVRs) and is the most potential target for vaccine design, because of their ability to induce a fast and longer-term immune response than other protein as it mediates virus entry and is a primary

determinant of cell tropism and pathogenesis [5, 7, 8, 9], therefore gained much popularity for researches [10, 11, 12]. In such situation of high emergence of new variants causing antigenic drift and low cross-protection between serotypes, and outbreaks of disease can occur even in vaccinated flocks [13, 14].

In Iraq IBV was detected in all Governorates in the North, Middle and South at very high rates [15, 16, 17, 18]. IBV variant 2 genotype, which showed extensive tissue tropism and implicated in kidney pathology [19] reported frequently in Iraq especially this decade [1, 16, 17, 20]. This variant is a major virus variant circulating in the Middle East countries and North Africa [1, 6, 16, 21-27]. The dissemination among the Middle East countries was attributed to movement of wild birds and intensive trade and uncontrolled movement of inhabitants and animal trade through the borders [16]. Variant 2 doses not respond to vaccines currently in use, challenge studies revealed that H120 vaccine provides poor protection [1, 28].

Vaccination is the most important method for controlling infectious bronchitis, and the genetic variation in the envelope and especially S1 is one of the main hurdles in design vaccine [1, 29]. Epitopes have become desirable candidates for vaccine design owing to their comparatively easy production, construction and chemical stability, in addition to the absence of infectious potential [30], and away from changes by mutations and recombination.

Epitope prediction of immunogens using Bioinformatics approaches and prediction tools reduces both the number of validation experiments and time for epitope detection [31]. The branch of Bioinformatics, namely Vaccinomics has been used to address the development of new vaccines [14], this approach is already validated in scientific community [32, 33, 34] as Bioinformatics becomes the central pillar of modern life science researches [35].

The aim of this study which carried out using Immunoinfocrmatics is to design vaccine against variant 2 which causes a large loss in economy of poultry industry in Iraq. As the designed vaccine should be ideal when provoke both humoral and cellular responses, both B cell and T cell epitopes (Cytotoxic T cell, and Helper T cell) were designed in order to cope with antigenic drift of IBV.

MATERIALS AND METHODS: Number of databases and software were used in this study:

NCBI / Protein, used to retrieve protein sequences.
https://www.ncbi.nlm.nih.gov/protein/

MEGA v.7 software: used for alignment and estimation of phylogeny [36].
http://69.36.184.213/mega.php

BioEdit: used for alignment [37].
http://www.mbio.ncsu.edu/BioEdit/bioedit.html

ExPASyProtParam tool: used for protein characterization.
https://web.expasy.org/protparam/

RAMPAGE software: used for estimation of protein Ramachandran plot.
http://mordred.bioc.cam.ac.uk/~rapper/rampage.php

Phyer2: used for protein modelling [38].
http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index

PEP-FOLD 3: used for prediction of short peptide 3D structure [39].
http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3

VaxiJen v2.0 software for prediction of antigenicity
http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html

IEDB Database: used for prediction of B- cell and T- cell epitopes and their characters [40].
http://www.iedb.org/

PyRx virtual screening Tool version 8: used for docking studies [41, 42].
https://pyrx.sourceforge.io/downloads

PyMol: used for vitalization [43].
https://pymol.org/2/

PDB database : used to retrieve pdb format of some proteins [44].

Multalin used for consensus sequence estimation [45].
http://multalin.toulouse.inra.fr/multalin/

RESULTS AND DISCUSSION: Vaccine is one of the most effective immunological intervention to control IBV infections [46], based on the pathogen used vaccine formulations might contain several proteins, some of them are unnecessary for induction of protective immunity [47], on the other hand vaccine should induce humoral and cellular

immune response, *i.e.*, triggers both B cells and T cells selectively [46]. For IBV spike protein (S1) is pairing with the cell receptors which is the key for infection and tropism, and to infect new host, it must adapt to the receptor of the new host either by mutations or recombination with other Corona viruses infecting the new host [5].

Sequence Retrieval: Sequences for S1 glyco-protein of the Middle East were collected from NCBI / GenBank database when have a clear declaration as variant 2, the proteins collected in FASTA format, using date of deposition in the database and the country, these sequences were aligned using ClustalW incorporated in MEGA package v7.0 and Neighbor Joining method for phylogenic tree was estimated, shown in **Fig. 1**.

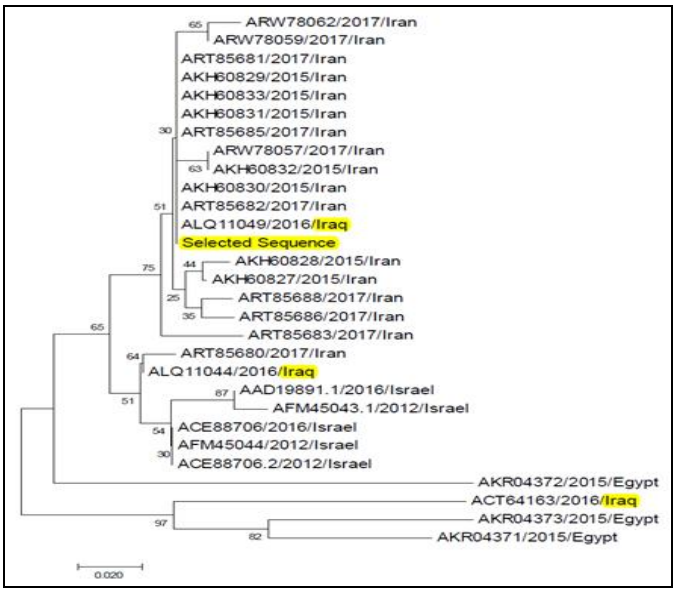


FIG. 1: PHYLOGENIC RELATION SHIP AMONG VARIANT 2 STRAINS CIRCULATING IN THE MIDDLE EAST

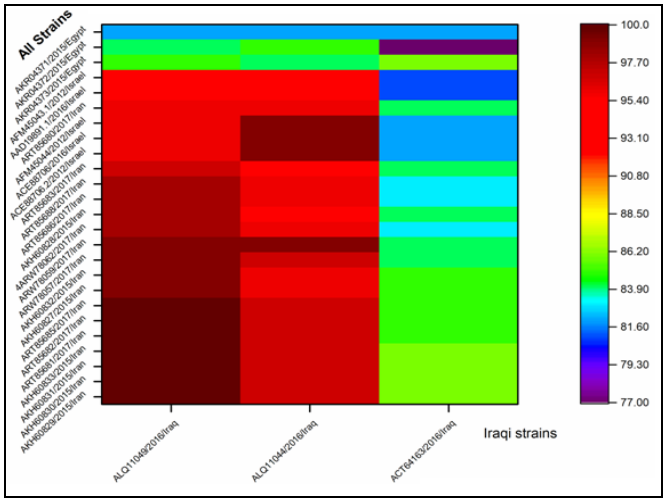


FIG. 2: SIMILARITY AMONG VARIANT 2 STRAINS *vs.* IRAQI STRAINS

The degree of similarity was estimated using BLAST (for 2 sequences), this is shown in **Fig. 2**.

The results indicate an absolute similarity of Iraqi strains with some strains from Iran, and high similarity with the Israeli strains, these results were expected as Iraq and Iran considered open trade market, in addition, it has been noted that only few amino acids substitutions resulting in 2% sequence divergence and can alter the serotypes and determining the pathogenic types [48], these changes of S1 subunit may be responsible for immune escape [49].

The retrieved sequences were subjected to VxiJen v2.0 to estimate the antigenic score as shown in **Table 1**.

TABLE 1: ANTIGENIC SCORE FOR VARIANT 2 STRAINS OF THE MIDDLE EAST

Strain	Antigenic score
RW78057/2017/Iran	0.9362
ART85685/2017/Iran	0.9187
ART85682/2017/Iran	0.9187
ART85681/2017/Iran	0.9187
ARW78059/2017/Iran	0.9054
ART85688/2017/Iran	0.9012
ARW78062/2017/Iran	0.8932
ART85680/2017/Iran	0.8872
AKH60832/2015/Iran	0.8723
ART85686/2017/Iran	0.8652
AKH60833/2015/Iran	0.8558
AKH60831/2015/Iran	0.8558
AKH60830/2015/Iran	0.8558
AKH60829/2015/Iran	0.8558
ALQ11049/2016/Iraq	0.8552
AKH60827/2015/Iran	0.8460
ART85683/2017/Iran	0.8458
ALQ11044/2016/Iraq	0.8372
AKH60828/2015/Iran	0.8170
AKR04372/2015/Egypt	0.6897
AKR04371/2015/Egypt	0.5925
ACE88706/2016/Israel	0.5850
ACE88706.2/2012/Israel	0.5850
AFM45044/2012/Israel	0.5825
AKR04373/2015/Egypt	0.5730
AAD19891.1/2016/Israel	0.5714
AFM45043.1/2012/Israel	0.5709
ACT64163/2016/Iraq	0.5495

The results revealed that all of them are antigenic as they exceed the threshold set (0.4).

Selected sequence was chosen from the variable regions, as the most variable region was found in HVR2 [1, 16, 50, 51], the selected region in this study shown in **Fig. 3**.

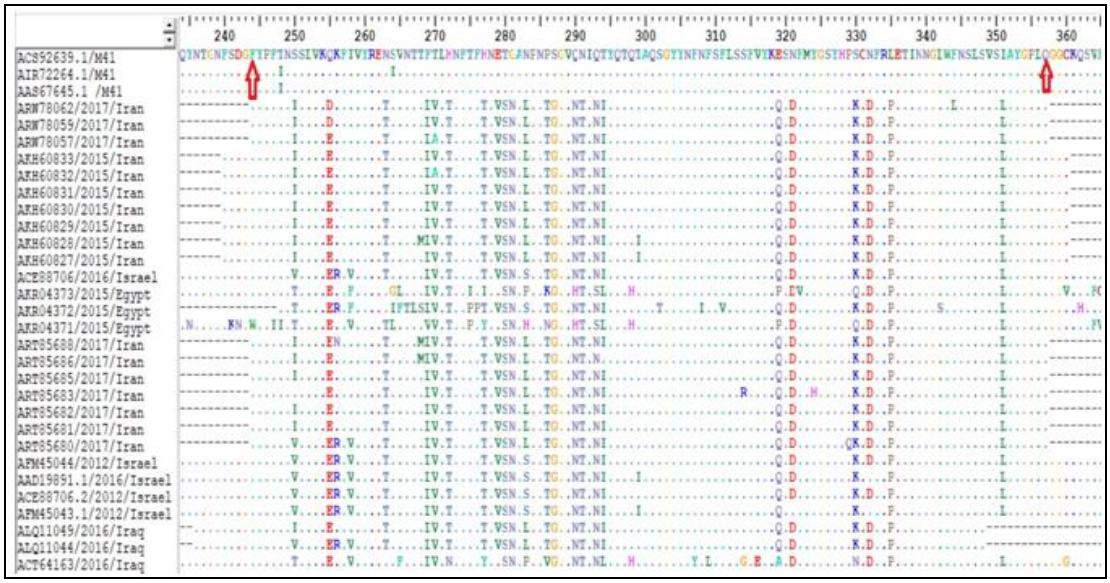


FIG. 3: THE SEGMENT OF S1 GLYCOPROTEIN SELECTED FOR STUDY

The sequence occupied the position 244-358 according to M41 strain (classical strain) numbering. From these sequences a consensus sequence was chosen using MutliAlin software and named selected protein in the rest of this study and used for further studies *i.e*., to predict B cell and T cell epitopes. The characterization of selected protein showed in **Table 2**.

TABLE 2: CHARACTERS OF SELECTED PROTEIN USED IN THIS INVESTIGATION (FYPFTNISLVKEK FIVYRETSVNTTLVLTNFTFTNVSNALPNTGGVNTI IYQTQTAQSGYYNFNFSFLSSFVYKQSDFMYGSYHP KCDFRPETINNGLWFNSLSVSLAYGPLQ)

Character	Value
Formula	$C_{604}H_{881}N_{145}O_{176}S_2$
Number of amino acids	114
Molecular weight	13053.63
No. of Negatively charged residues (Asp+Glu)	5
No. of Positively charged residues (Arg+Lys)	6
Isoelectric point (PI)	8.03
Instability index	32.18
Aliphatic index	70.88
Grand average of hydropathicity (GRAVY)	-0.137

Table 2 Expasy characters

The results showed that the protein is positively charged as the residues (Arg + Lys) more than the negatively charged residues (Arg + Glu), the isoelectric point (8.04), *i.e*., slightly basic demonstrating that the protein is nonallergenic [52], the GRAVY hydropathicity with negative value (-0.137) indicates that the protein is hydrophilic, other characters point to be stable with aliphatic nature. The antigenic score of selected protein is high (0.9041), Ramachandran plot was carried out to indicate the feasibility or fitness of the protein as shown in **Fig. 4**.

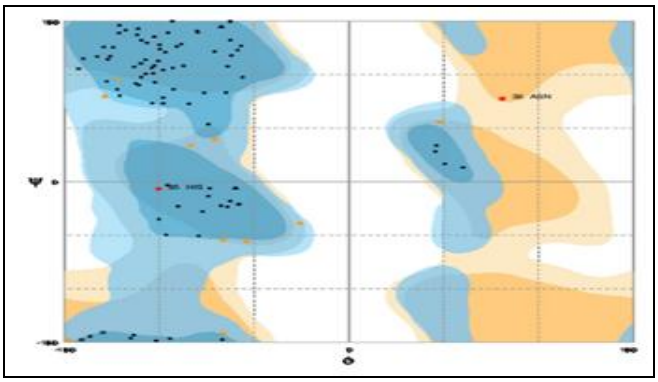


FIG. 4: RAMACHANDRAN PLOT OF SELECTED PROTEIN

These results indicate that 87% of the residues are in the allowed region. 3D structure of the protein was estimated using Phyer 2 software **Fig. 5**, it appears with rich loops and β-turn structures.

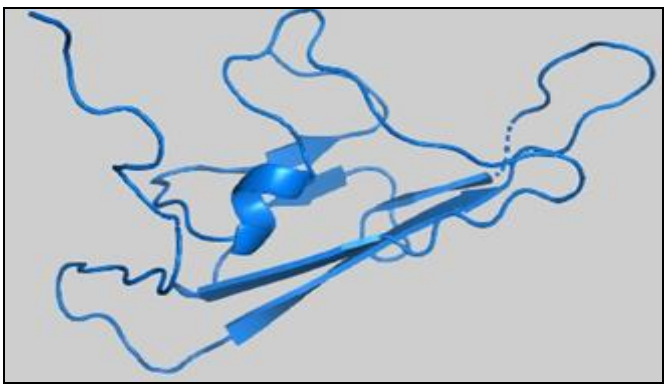
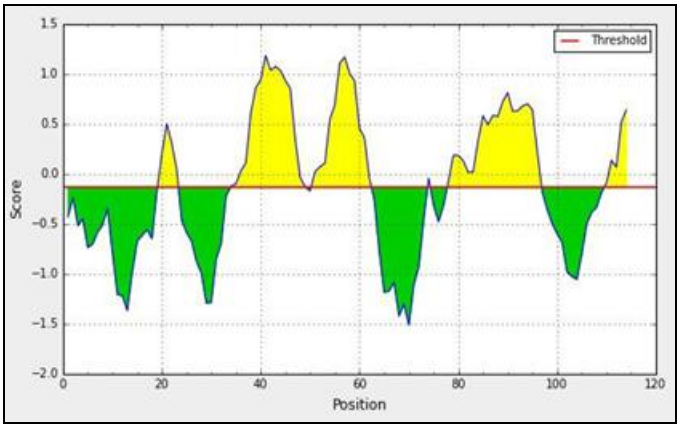


FIG. 5: 3D STRUCTURE OF PROTEIN ESTIMATED BY USING PHYER 2 SERVER

Epitope Predictions: Epitope is an amino acids stretch binds to antibody , located on the surface of the antigen [46], interestingly S1 protein of IBV (about 520 amino acids) contains different immune epitopes responsible for both antibodies and induction of cell based immunity responses (CTL and Helper cells), thus playing as viral antigenic determinants [54]. In this neutralizing antibodies are important in removing freely circulating IBV, whereas CTL response is crucial for control and clearance of virus infected cells [55]. To achieve this, stimulation of both humoral (B cells) and CMI (Cytotoxic and Helper cells) is considered very essential for any candidate vaccine [56].

B Cell Epitopes: Continuous / linear epitope was predicted using the selected protein sequence and IEDB / BepiPred, The results gave three candidate epitope shown in **Fig 6**.



Position	Sequence	Length	Antigenic score
20-23	TSVN	4	
34-49	TNVSNALPNTGGVNTI	16	0.2662
51-62	IYQTQTAQSGYY	12	0.5945
78-96	DFMYGSYHPKCDFRPETIN	19	1.4289

FIG. 6: PREDICTED B CELL EPITOPES IN THE SELECTED PROTEIN

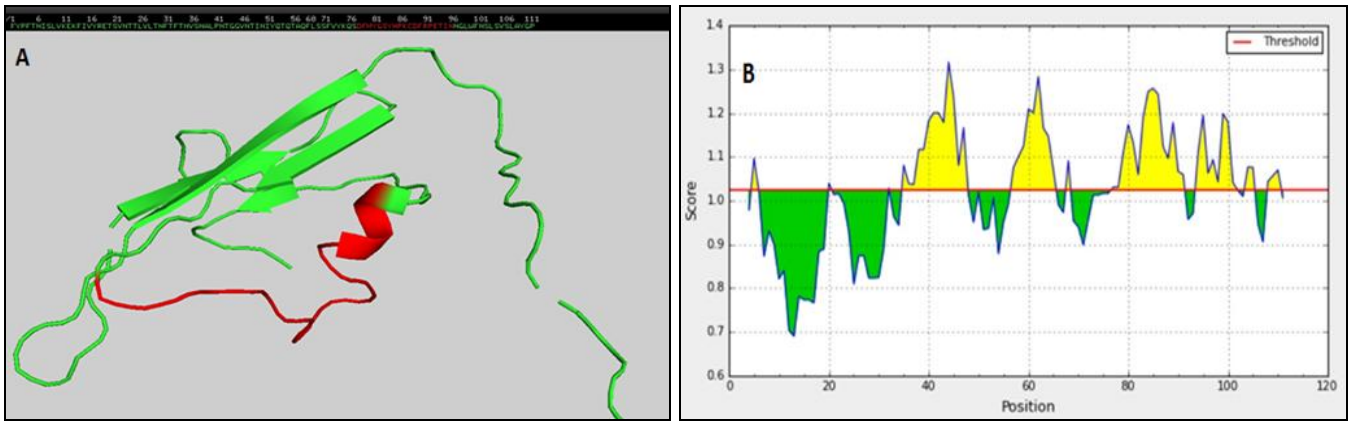


FIG. 7: CHARACTERS OF SELECTED B CELL EPITOPE, THE POSITION IN *β*-TURN

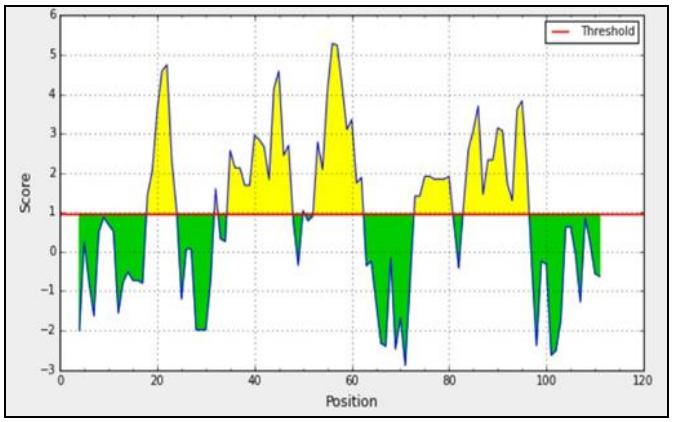


FIG. 8: PARKER HYDROPHILICITY PREDICTION OF B CELL EPITOPES

Only epitopes present on the outer surface were chosen, the results showed only one epitope is suitable, this at the position 78-96, with antigenicity score 1.4289 (VaxiJen at cut off value 0.4). The epitope belongs to *β*-turn of the protein **Fig. 7A**, **B**. As the B cell epitope on the surface, it exhibits a hydrophilic nature as shown in **Fig. 8**.

T Cell Epitopes: Since the immune response of T cells is long-lasting compared to B cells, where the antigen can easily escape the antibody memory response [47], the T cell epitopes were predicted. CTL epitopes were predicted using IEDB / TepiTool, attached to all alleles of MHC I (HLA-A, -B, -C) available in the database, lots of epitopes resulted, these were subjected to filtration, mainly using proteasomal and TAP scores to distinguish the epitope from nonepitope [57, 58], percentile less than 1 and binding affinity less than 200 nm, only four epitopes are satisfied the restriction parameters, and are able to attach to many HLA alleles, **Table 3**.

TABLE 3: PROTEASOME SCORE AND TAP SCORE OF SELECTED T CELL EPITOPE AND THE ALLELES INTACT WITH THEM

Position	Sequence	Proteasome Score	TAP Score	Alleles
53-61	QTQTAQSGY	1.29	1.23	HLAA*01:01,HLAA*02:01,HLAA*03:01 HLAA*11:01,HLAA*23:01,HLAA*24:02 HLAA*25:01,HLAA*26:01,HLAA*30:01 HLAA*31:01 HLAB*0:01,HLAB*07:02,HLAB*08:01 HLAB*13:01,HLAB*14:02,HLAB*15:01 HLAB*15:02,HLAB*15:25,HLAB*35:01 HLAB*35:03,HLAB*37:01,HLAB*38:23 HLAB*44:02,HLAB*46:01,HLAB*48:01 HLAB*49:01,HLAB*51:01,HLAB*52:01 HLAB*55:01,HLAB*56:01,HLAB*58:01 HLAC*01:02,HLAC*02:09,HLAC*03:04 HLAC*04:01,HLAC*06:02,HLAC*07:04 HLAC*12:02,HLAC*12:03,HLAC*14:02 HLAC*15:02,HLAC*16:01,HLAC*17:01
64-72	FNFSFLSSF	1.12	1.10	HLAA*01:01,HLAA*02:01,HLAA*03:01 HLAA*11:01,HLAA*23:01,HLAA*24:02 HLAA*25:01,HLAA*26:01,HLAA*29:02 HLAA*30:01,HLAA*31:01,HLAA*32:01 HLAA*33:03,HLAA*74:01 HLAB*07:02,HLAB*08:01,HLAB*13:01 HLAB*14:02,HLAB*15:02,HLAB*35:01 HLAB*37:01,HLAB*38:23,HLAB*40:01 HLAB*44:02,HLAB*46:01,HLAB*48:01 HLAB*49:01,HLAB*51:01,HLAB*52:01 HLAB*55:01,HLAB*56:01,HLAB*58:01 HLAC*02:09,HLAC*04:01,HLAC*06:02 HLAC*07:04,HLAC*12:02,HLAC*12:03 HLAC*14:02,HLAC*14:02,HLAC*15:02 HLAC*16:01,HLAC*17:01,HLAC*3:04
66-74	FSFLSSFVY	1.44	1.36	HLAA*01:01,HLAA*02:01,HLAA*03:01 HLAA*23:01,HLAA*24:02,HLAA*26:01 HLAA*29:02,HLAA*30:01,HLAA*30:02 HLAA*31:01,HLAA*32:01,HLAA*32:01 HLAA*33:03,HLAA*68:02,HLAA*74:01 HLAB*07:02,HLAB*08:01,HLAB*13:01 HLAB*14:02,HLAB*15:01,HLAB*15:02 HLAB*15:25,HLAB*35:01,HLAB*35:03 HLAB*37:01,HLAB*38:23,HLAB*40:01 HLAB*44:02,HLAB*46:01,HLAB*48:01 HLAB*49:01,HLAB*51:01,HLAB*52:01 HLAB*55:01,HLAB*56:01,HLAB*58:01 HLAC*01:02,HLAC*02:09,HLAC*03:04 HLAC*04:01,HLAC*06:02,HLAC*07:04 HLAC*12:02,HLAC*12:03,HLAC*14:02 HLAC*16:01,HLAC*17:01
102-110	NSLSVSLAY	1.39	1.27	HLAA*01:01,HLAA*02:01,HLAA*03:01 HLAA*11:01,HLAA*23:01,HLAA*24:02 HLAA*25:01,HLAA*26:01,HLAA*29:02 HLAA*30:01,HLAA*30:02,HLAA*31:01 HLAA*32:01,HLAA*33:03,HLAA*68:02 HLAA*74:01,HLAB*07:02 HLAB*08:01,HLAB*13:01,HLAB*14:02 HLAB*15:01,HLAB*15:02,HLAB*15:25 HLAB*35:01,HLAB*35:03,HLAB*37:01 HLAB*38:23,HLAB*40:01,HLAB*44:02 HLAB*48:01,HLAB*49:01,HLAB*51:01 HLAB*51:01,HLAB*52:01,HLAB*55:01 HLAB*56:01,HLAB*58:01 HLAC*01:02,HLAC*02:09,HLAC*03:04 HLAC*04:01,HLAC*06:02,HLAC*07:04 HLAC*12:02,HLAC*12:03,HLAC*14:02 HLAC*15:02,HLAC*16:01,HLAC*17:01

Cell responses of cytotoxic (CD8) and helper cells (CD4) play a major role in antiviral immunity (as mentioned previously), so designing of vaccines provoking T cell is much promising [47], and investigation of binding affinity of antigenic epitope to MHC molecules is the goal for predicting epitopes [30] as the processing and presentation of MHC I of antigen is a key mechanism in surveilling and recognizing viral particles by immune system of the infected cells [53], on the other hand, epitope has high binding affinity to several alleles tends to be potential candidate for epitope based vaccine design , and this verified by the selected epitopes mentioned above. T helper cell (CD4) epitopes were predicted as well using IEDB / TepiTool, with percentile less than 1, and binding affinity less than 200nM, and all MHC II alleles (HLA-DR, -DP, -DQ) available in the database, 8 epitopes with 15mer were resulted, all of them have antigenic score above the VaxiJen threshold (0.4), these are shown in **Table 4**.

TABLE 4: EPITOPES FOR T HELPER CELLS AND THE REACTING HLAs MOLECULES

Position	Sequence	Alleles
60-74	GYYNFNFSFLSSFVY	HLA-DPA1*01/DPB1*04:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*11:14,HLA-DRB1*11:20,HLA-DRB1*11:14,HLA-DRB1*11:20
13-27	KFIVYRETSVNTTLV	HLA-DRB1*15:06,HLA-DRB1*04:02,HLA-DRB1*08:01,HLA-DRB1*08:04,HLA-DRB1*08:06,HLA-DRB1*08:13,HLA-DRB1*08:17
28-42	LTNFTFTNVSNALPN	HLA-DRB1*04:03, HLA-DRB1*04:04, HLA-DRB1*04:06, HLA-DRB1*04:07, HLA-DRB1*04:09, HLA-DRB1*04:14, HLA-DRB1*04:16, HLA-DRB1*04:17 HLA-DRB1*04:19,HLA-DRB1*04:45,HLA-DRB1*04:46,HLA-DRB1*04:47,HLA-DRB1*04:48,HLA-DRB1*04:49,HLA-DRB1*04:50,HLA-DRB1*04:52,HLA-DRB1*04:56,HLA-DRB1*04:57,HLA-DRB1*04:60
43-57	TGGVNTINIYQTQTA	HLA-DRB1*04:02, HLA-DRB1*04:08,HLA-DRB1*04:23,HLA-DRB1*08:02,HLA-DRB1*08:04,HLA-DRB1*08:06,HLA-DRB1*08:13 HLA-DRB1*11:02,HLA-DRB1*11:21,HLA-DRB1*11:02,HLA-DRB1*11:21,HLA-DRB1*15:06
48-62	TINIYQTQTAQSGYY	HLA-DRB1*04:02,HLA-DRB1*04:08,HLA-DRB1*04:21,HLA-DRB1*04:23,HLA-DRB1*08:02,HLA-DRB1*08:04,HLA-DRB1*08:06,HLA-DRB1*08:13,HLA-DRB1*11:02,HLA-DRB1*11:21,HLA-DRB1*11:02,HLA-DRB1*11:21
62-76	YNFNFSFLSSFVYKQ	HLA-DRB1*03:05,HLA-DRB1*15:01,HLA-DRB1*15:08,HLA-DRB1*15:11,HLA-DRB1*15:14,HLA-DRB1*15:15,HLA-DRB1*15:19,HLA-DRB1*15:26,HLA-DRB1*15:29,HLA-DRB1*15:30,HLA-DRB1*15:31,HLA-DRB1*15:38,HLA-DRB1*15:39,HLA-DRB1*15:44,HLA-DRB1*15:47
96-110	NNGLWFNSLSVSLAY	HLA-DRB1*04:01,HLA-DRB1*04:03,HLA-DRB1*04:05,HLA-DRB1*04:44,HLA-DRB1*04:45,HLA-DRB1*04:46,HLA-DRB1*04:47,HLA-DRB1*04:48,HLA-DRB1*04:49,HLA-DRB1*04:50,HLA-DRB1*04:51,HLA-DRB1*04:52,HLA-DRB1*04:54,HLA-DRB1*04:57,HLA-DRB1*04:60,HLA-DRB1*04:61,HLA-DRB1*04:44,HLA-DRB1*08:13
97-111	NGLWFNSLSVSLAYG	HLA-DRB1*01:01,HLA-DRB1*01:13,HLA-DRB1*01:14,HLA-DRB1*01:17,HLA-DRB1*04:06,HLA-DRB1*04:07,HLA-DRB1*04:08,HLA-DRB1*04:09,HLA-DRB1*04:16,HLA-DRB1*04:17,HLA-DRB1*04:19,HLA-DRB1*01:21,HLA-DRB1*01:24,HLA-DRB1*01:29,HLA-DRB1*09:01,HLA-DRB1*10:01,HLA-DRB1*10:03,HLA-DRB1*09:01,HLA-DRB1*10:01,HLA-DRB1*10:03,HLA-DRB1*14:10

All the epitopes were BLASTed using NCBI/ BLAST and were found to for spike protein of IBV, in addition their allergenicity were also estimated and found in agreement with FAO/WHO regulations.

Docking Studies: Molecular docking is a key tool in structural molecular biology and computer - assisted drug design, and binding of immunogenic epitopes to MHC molecules makes reliable predictions of epitopes that minimizes the experimental efforts needed to identify new epitopes to be used in vaccine design [35], at the same time little information about MHC alleles in poultry are available [59], while MHCs of several organisms are supported by tools of IEDB, chicken not among them, and there is no database or software available to calculate the binding affinity of epitope with specific chicken MHC I or MHCII alleles [47], but the attitude used by researchers is to use human MHC molecules (HLAs) at the computational stages, and this confirmed by molecular docking with known chicken MHC molecules, This depending on facts that several studies suggest similarity between HLA alleles and chicken MHC [60-63], and since the MHC molecules are among the most polymorphic proteins in higher vertebrates, and there are more than 6000 alleles for MHC I and MHC II, this necessities developing of Bioinformatics tools to deal with [30].

MHC I alleles (HLA-A, -B, -C) were chosen instead of B-F chicken alleles at a computational prediction stage, since B-F molecules have been structurally and functionally linked to mammalian MHC I and involved in antigen presentation to CD8 T lymphocytes [64]. Among, the B-F molecules BF2*21:01 is predominantly expressed and exhibits a promiscuous peptide binding affinity, so it performs a uniqueness in flexibility for binding different peptides [63], the other MHC I allele BF2.0401, is positively charged molecule and practically can bind a variety of epitope peptides [65].

All the predicted epitopes were subjected to PEP-FOLD 3.0 web-based servers for 3D structure conversion in order to analyze the interactions with HLA molecules. The epitopes of **Table 3** were docked with BF2*21:01 (pdb ID 3bev) and BF2.0401 (pdb ID 4g42) using PyRx Virtual Screening Tool version 8, the Grid extended to cover the protein molecule, epitope used as ligand, the parameters for docking each epitope shown in **Table 5**.

TABLE 5: THE PARAMETERS USED FOR DOCKING PROCESS

Position	Sequence	Δ G kal/mol	RMSD value
	3bev		
53-61	QTQTAQSGY	-8.3	0
64-72	FNFSFLSSF	-8.1	0
66-74	FSFLSSFVY	-9.2	0
102-110	NSLSVSLAY	-7.2	0
	4g42		
53-61	QTQTAQSGY	-8.9	0
64-72	FNFSFLSSF	-8.8	0
66-74	FSFLSSFVY	-8.9	0
102-110	NSLSVSLAY	-8.0	0

Visualization shown in **Fig. 9A** for 3bev allele and **Fig. 9B** for 4g42 allele.

The 3D structure of chicken B-L (MHC II molecules) and human HLA-DR1 are similar at 66%, the former has more polymorphic sites, probably to compensate responding to wide varieties of pathogens in chickens [61]. In addition MHC II binding groove is open from both ends unlike MHC I binding groove which is closed, therefore longer peptides (more than 15mer) can fit in this groove [35], this variability in binding makes prediction / docking difficult and less accurate [35], and since there is no pdb structure for chicken MHC II molecules available, this docking was unable to perform.

Nowadays, it seems never-ending race with IBV as new variants are continuously emerging in major poultry production countries, and most vaccines are based on B cell immunity which can be escaped, but recently T cell (CD8) immunity generates a strong immune response is practiced [14]. On the other hand, many vaccines trails are currently being conducted worldwide , but they fail to reach phase III, this indicates a gap between the early stages trials (phase I and phase II) and the efficacy trail (phase III) [31]. And it is well known that the core goal behind all vaccinations is to initiate an immune response in a quicker fashion than the pathogen itself [30].

CONCLUSION: In conclusion peptide (epitope) vaccine is strongly supersedes the conventional vaccines as it design to cover variant virulent mutated strains, which will reduce the recurrent

outbreaks in vaccinated flocks and their huge accompanied economical loss to the minimum. In addition epitope vaccine helps in exclusion of suppressive epitopes and exclude the emergence of new variants by mutation of the viral genome and recombination with other viruses. So in this approach it is possible to incorporate several epitope peptides directed against different viruses or multiple virus strains into single delivery system, with view to induce broad and specific immune response in a single administration [55, 66].

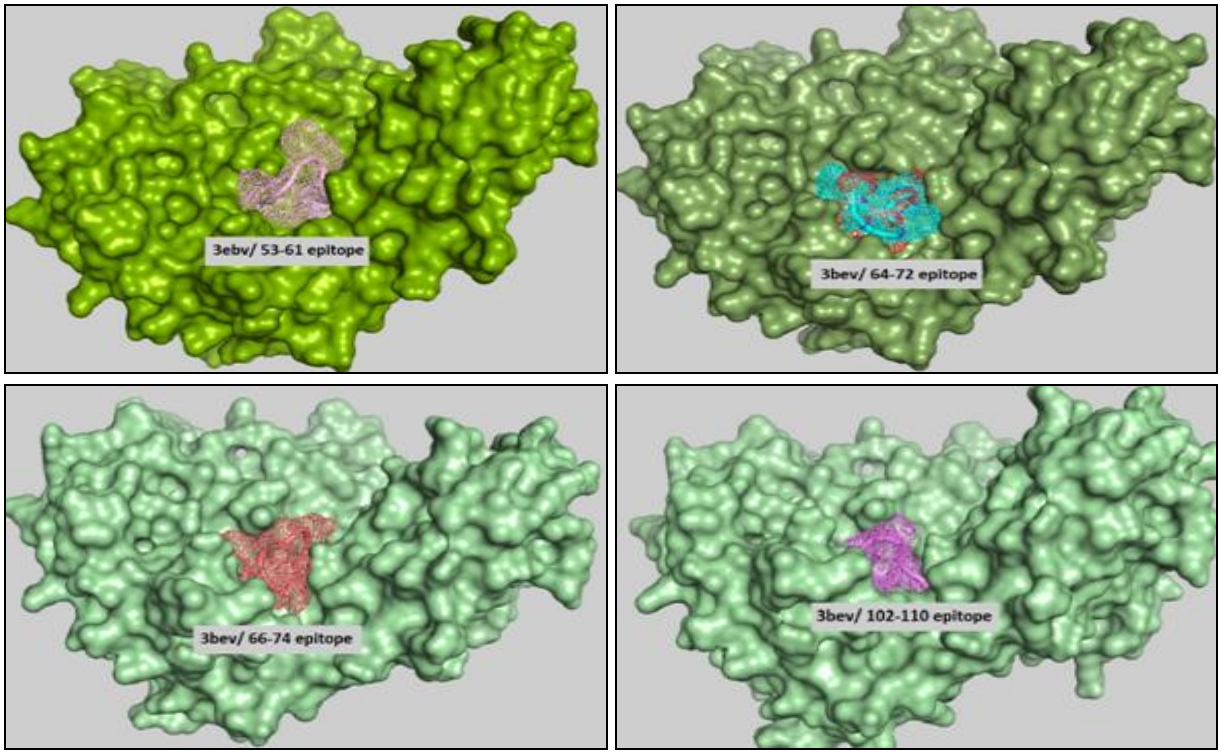


FIG. 9A: DOCKING WITH BF2*21:01 (CHICKEN MHC I MOLECULE)

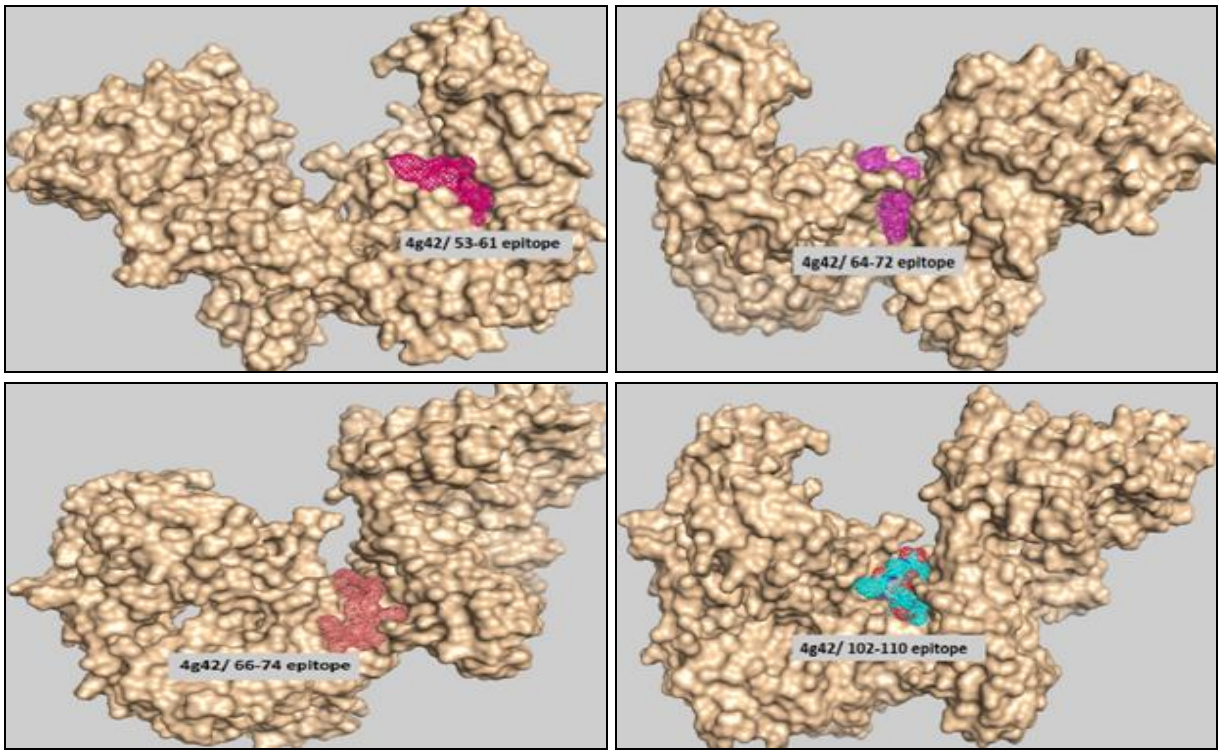


FIG. 9B: DOCKING WITH BF2.0401 (CHICKEN MHC I MOLECULE)

Epitope based vaccine designing is more promising, as the conventional vaccines lies on the response induced by the natural immunogen which are not optimal. In this study the selected protein was used for epitope prediction supposed to work with all strains. And this can be combined with global types to generate universal vaccine. Overlapped epitopes predicted in this study could introduce the possibility of antigen presentation to immune cells *via* both MHC I (B-F) and MHC II(B-L) pathways.

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