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DESIGNING OF MLTI-EPITOPE VACCINE BASED ON VACCINE STRAINS AGAINST IRAQI AND VARIANT 2 INFECTIOUS BRONCHITIS VIRAL STRAINS

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ABSTRACT: Infectious bronchitis disease is a contiguous disease in poultry, treated mainly by vaccination which is failed at high rate due to high variation of the Infectious Bronchitis Virus (IBV). Epitope –based vaccine as promising approach was used. In this study epitopes were derived from consensus sequence of vaccine strains used in Iraq, characterized and some of them docked with chicken MHC I molecules. Two B cell epitopes were obtained:51-59 (THTAQSGYY), 76-84(FMYGSYHPS) which satisfied the required criteria. Five CLT epitopes: 22-30 (TLELTNFTF), 57-65(GYYNFNFSF), 68-76(SFVYKESNF), 73-81(ESNFMYGSY), 99-107(NSLSVSLAY) were obtained according to acceptable criteria and docked very well with MHC I molecules of chickens . Six epitopes for helper T cell were obtained: 56-70(SGYYNFNFSFLSSFV), 57-71(GYYNFN FSFLSSFVY), 58-72(YYNFNFSFLSSFVYK), 61-75(FNFSFLSSFVYKESN) ,63-77(FSFLSSF VYKESNFM) ,64-78(SFLSSFVYKESNFMY), they react with many HLA alleles at high affinity.

KEYWORDS : Multi-epitope vaccine , Variant 2 , Iraq , Vaccine Failure , Immunoinformatics

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1.INTRODUCTION

Infectious bronchitis virus (IBV) is an RNA virus presents in all countries with an intensive poultry industry, causing infectious bronchitis with incidence of infection approaching 100% in most locations. The virus with great ability for mutation and recombination, thus capable for generation of new strains that are difficult to control, the higher changing is distinguish characteristic of this virus among other coronaviruses [1,2,3] . The tendency for recombination and high mutation rates may allow the virus for adaptation to new hosts and ecological niches and to escape immune system [4,5,6,7]. Vaccination is the most important method to control the viral infection, but it is only partially successful due to continual emergence of new antigenic variants [8] . This necessities development of safer and more effective vaccines for practical control of IBV [9] . Spike glycoprotein, specially S1 (surface structural protein) of the virus could represent an important target in prevention of IB outbreaks [10]. The vaccines used around the world are mainly prepared from certain strains such as H120 [11], this strain is able to spread extensively among broilers, this implies that this vaccine strain might be able to become endemically present in poultry population [12], but the disadvantages of IBV H120 vaccine might be a predispose for colibacillosis under high *Escherichia coli* pressure [13,14,]. H strains have shown a rare ability for cross-protection against heterologous serotypes [15], but cannot protect variant 2 infections [16,17]. However, it has been shown that vaccination with selected antigenically distinct strains can result in cross-protection against many different IBV strains [18,19]. In Iraq, IBV is highly prevalent and especially variant 2 sharing the other Middle East countries [20,21], vaccines are used (sometimes without official authorization) , and those are H120, H52,4/91, D274 . In all cases there is insufficient protection against IBV infections using conventional vaccine due to many reasons [1,22,23,24,25,26,27]. In addition, the use of vaccines especially the live attenuated type and in the presence of multiple infections with different IBV serotypes contributes to recombination process that favors the emergence of new IBV variants [28]. So peptide vaccine was found to be an effective and powerful approach [9]. The peptide vaccines are candidate owing to their comparatively easy production , construction , chemical stability , absence of infection potential [29,30] and no way for changing by mutations or recombination processes. Epitope based vaccine design is more promising ,as the conventional vaccine approach lies on responses induced by natural immunogen which may be not optimal [27]. Immunoinformatics makes the epitope based vaccine possible by identification of relevant epitopes and significantly improve the vaccine design and development , since reduces the number of validation experiments , cost and time . The *In Silico* /Bioinformatics approach has been extended to poultry and accepted in the scientific communities [28,29,30,31,32]. The aim of this research is based on Immunoinformatics to predict and select epitopes using S1 glycoprotein

(partial Sequence) the most important immunogen of the virus, the prediction of vaccine depends on the Iraqi strains prevalent in the country and variant 2 which is widely prevalent as well .

2. MATERIALS AND METHODS

This study is absolutely dry lab work i.e., In Silico study . Different databases and servers or software were used: NCBI / Protein, used to retrieve protein sequences, NCBI/BLASTx2, used to estimate similarities and existence of epitopes. MEGA v.7 software, used for alignment and estimation of phylogeny [33]. BioEdit , used for alignment and Entropy measuring [34] . ExPASy: ProtParam tool used for protein characterization. RAMPAGE software , used for estimation of protein Ramachandran plot . Phyer2: used for protein modelling [35], PEP-FOLD 3: used for prediction of short peptide 3D structure [36]. VaxiJen v2.0 software for prediction of antigenicity. IEDB Database, used for prediction of B- cell epitopes and their characters [37]. PyRx virtual screening Tool version 8 , used for docking studies [38,39]. PyMOL, used for vitalization . PDB database : used to retrieve pdb format of some proteins [40]. Multalin used for consensus sequence estimation [41]. TMHMM Server v. 2.0, used to check the presence of epitopes [42]. NetMHCpan version 4.0 :used for prediction MHC I , cytotoxic T cell epitopes and their attached alleles [43] .TAPPred software : used to estimate Cytotoxic T cell epitopes Tap Score [44] . NetMHCIIpan 3.2 Server : used for prediction MHC II , helper T cell epitopes [45] . EpiTop 1.0 : used for prediction MHC II , helper T cell epitopes [46] .

3. RESULTS AND DISCUSSION

Vaccine is generally considered to be the most effective method for preventing infectious diseases [47] , and the core mechanism behind all vaccinations is the ability of vaccine to initiate an immune response in a quicker mode than the pathogen itself [30] . In addition the ideal vaccine should elicit humoral and cellular response that can trigger the B cells and T cells selectively [48].On the other hand the major problem in viral vaccine design is the difficulty in provoking immune response against antigenically different strains [49], this is true for RNA viruses with high mutation rates such as IBV [50,51], so the most important step in designing of cross-protective vaccine against IBV is to target the conserved epitopes of different serotypes [9]. For IBV , the S1 glycoprotein has proven to be critical for antigenic neutralization , hemagglutination and cell/tissue tropism determination , it contains the primarily epitopes recognized by host immune system , and the diversity of S1 protein sequence primarily accounts for serotype variations [41,52,53] . It is known that S1 protein is sufficient to induce good protective immunity [52,54]. It contains different immune epitopes responsible for both antibodies and cytotoxic T cells (CTL)-viral antigenic determinants [55]. Monoclonal antibody analysis has revealed that many of the amino acids involved in the formation of virus neutralizing antibody-inducing epitopes , that occur in the hypervariable regions especially

in 1st and 3rd quarter of the linear S1 polypeptide [56,57], therefore the S1 sequences were taken in consideration in designing novel control strategies [58].

S1 glycoprotein sequences: According to above discussion, the S1 protein sequences were retrieved (most of them partial sequences) from NCBI. Thirty six sequences belong to Iraqi sources and 28 sequences for variant 2 , these two groups were studied and investigated previously [59,60]. Vaccine strain S1 sequences were retrieved as well when there is a clear declare as vaccine strain, and only the vaccine strains used in Iraq were used. The similarity between Iraqi and variant 2 strains and vaccine strains is shown in Fig 1

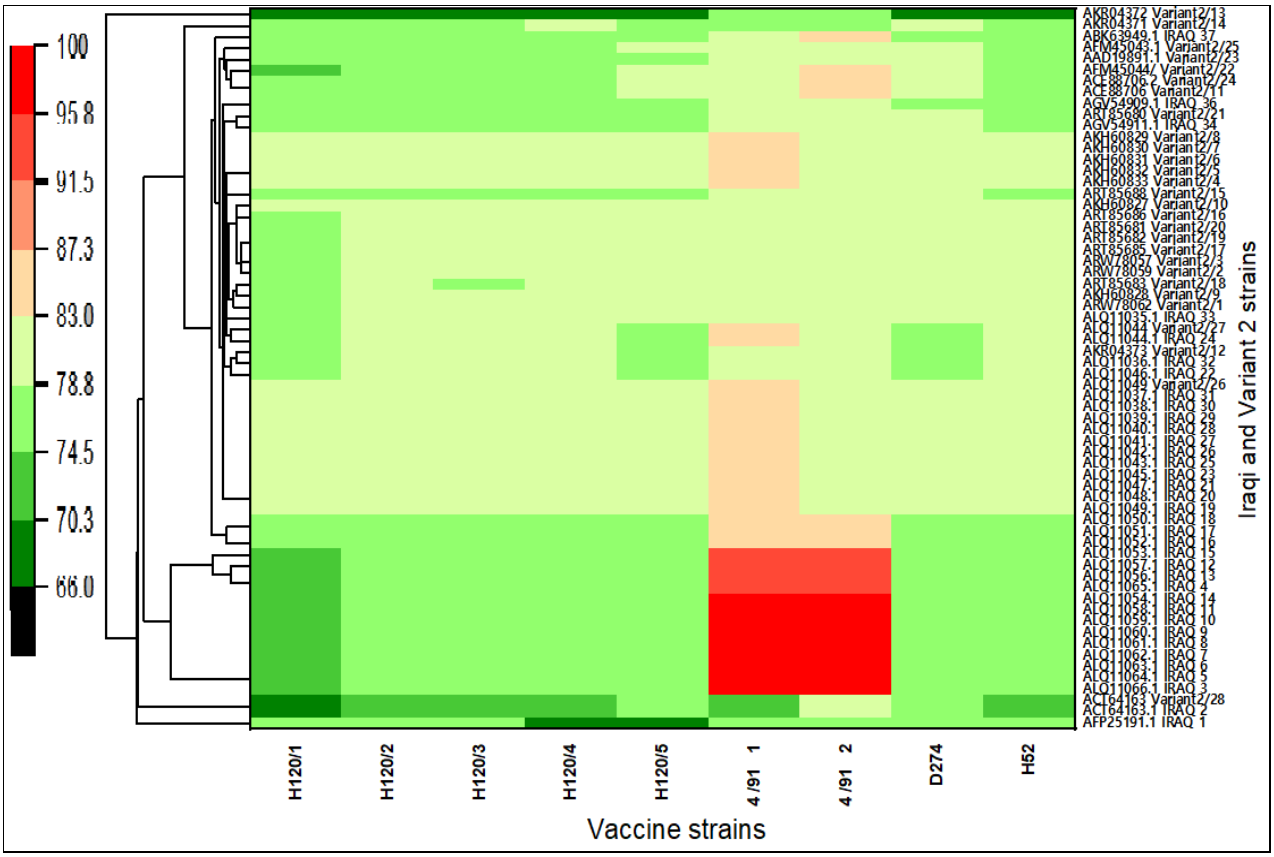


Fig 1: Similarity between vaccine strains and Iraq ,Variant2 strains (Test strains)

The Figure shows that only vaccine strain 4/91 (1and 2)have 100% similarity with some Iraqi strains , and most sequences vary in their similarities , which can be low to less than 70%, other studies recorded such inferior similarity especially for different strains of variant 2 . For H120 this strain similarity was 72.9-76.5% , other studies showed a similarity range 78.8-80% [61]. In addition the Iraqi strain (Sul/01/09) differs at 27-28% from the vaccine strains 4/91 and H120 used in the country [62]. It has been estimated that analysis of different parts of S1 protein can result in different level of homology [23], and there is increasing evidences that only a few amino acids differences amongst S protein are sufficient to have detrimental impact on cross protection [5,36]. In other viruses the difference in S protein as few as one or two amino acids as in the porcine transmissible

gastroenteritis (TGEV) determine whether the virus is enteropathogenic or non-pathogenic in pigs [5,36]. So it might expect that cross protection tends to diminish as the degree of amino acids identity/similarity between S1 protein of two IBV strains decreases . These reasons explain that heterogeneity with vaccine strains behind the poor vaccination performance or even vaccine failure and disease outbreaks in this area [61,62] . On other hand , and according to vaccines used in Iraq , it has been found that 4/91 vaccines are contributing to emergence of new variants in the field in chicks as shown in chinses studies [63]. In addition evidences have shown that despite the effort to reduce viral virulence using 52nd and 120th passage to produce H52 and H120 IBV vaccines , these vaccines potentially cause considerable pathology of trachea and may lead to sever outbreaks in field [64] . In an attempt to prepare epitope vaccine depending on S1 protein sequences, the retrieved sequences of the Iraqi strains and variant 2 sequences were aligned and the entropy for changeable amino acids was estimated as shown in Fig 2

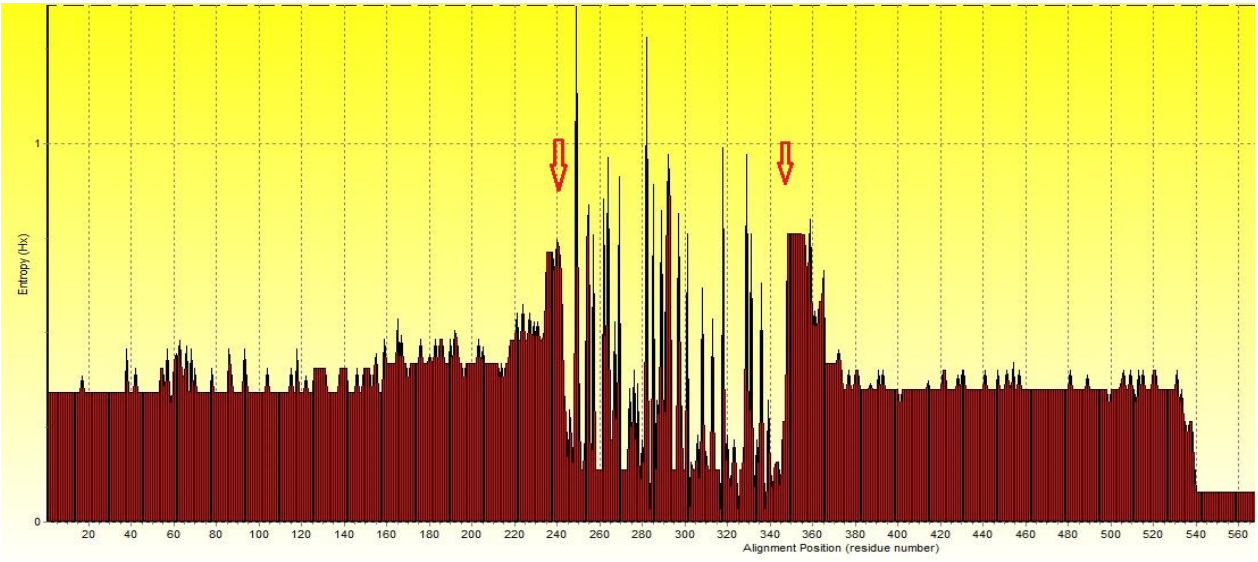


Fig 2 : Entropy of S1 protein amino acids Sequences of test strains

The results of entropy of amino acids sequences revealed that S1 gene has the largest variation, so the genetic diversity and viral evolution of IBV are mainly monitored by analysis of S1 gene , however , pathogenicity is associated with spike gene (mainly) as well as outside the spike gene [63] . For entropy, it is known that the higher the peak is the greater entropy is , indicating the higher variation frequency of amino acids sites . As entropy values greater than 0.4 indicates that the corresponding amino acids site is not conserved and is prone by substitution, this is the results of positive selection, since deleterious mutants are reduced, while the promoting advantageous mutation are fixed, this may correlate with structurally or functionally important residues [63] .

To find the suitable region for deriving epitopes , all the Iraqi strains and variant 2 strains were aligned and depending on the results of Fig 2 ,consensus amino acids sequence was suggested from

these datasets [30] which ranged from position 247-355 to be used for prediction of B cell and T cell epitopes within the S1 glycoprotein . The consensus sequence (named selected protein) was aligned with S1 protein of vaccine strains and Neighbor joining relationship was estimated using MEGA software 7.0 as shown in Fig 3

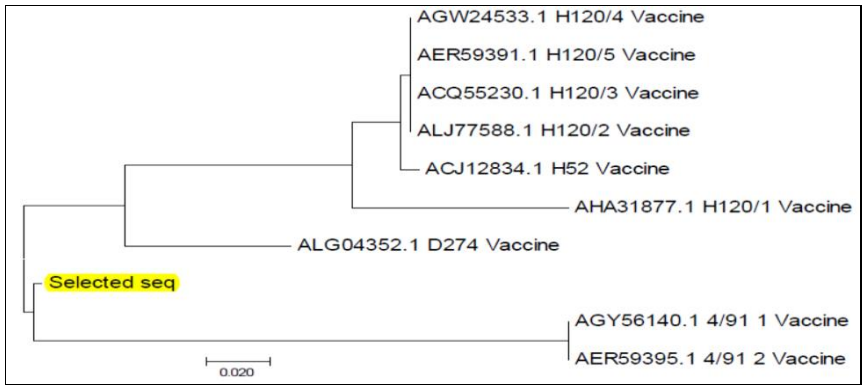


Fig 3 : Phylogenic relationship among selected protein and S1 protein of selected vaccine strains

Properties of selected protein : The selected protein/sequence has the following sequence residues :(FTNSSLVKQKFIVYRESSVNTTLELTNFTFTNVTSASPNPSGVNTIQLYQTHTAQS GYYNFNFSFLSSFVYKESNFMYGSYHPSCNFRLETINNGLWFNSLSVSLAYGP), this sequence showed 88-93% similarity with vaccine strains , and 87-92% similarity with the Iraqi and variant 2 sequences , except for variant 2/8 the similarity lowered to 84% . The antigenic score is 0.8071 using VaxiJen v2.0 software at threshold 0.4 . the other properties of the selected protein are shown in Table 1

Table 1 : Characters of selected protein

Character	**Value**
Formula	C565H824N140O171S2
Number of amino acids	109
Molecular weight	12377.71
No. of Negatively charged residues (Asp+Glu)	4
No. of Positively charged residues (Arg+Lys)	5
Isoelectric point (PI)	8.07
Instability index	20.96
Aliphatic index	64.31
Grand average of hydropathicity (GRAVY)	-0.219

The results indicate that the protein is positively charged as the residues (Arg+Lys) more than the negatively charged residues (Arg+Glu), the isoelectric point (8.07), i.e., slightly basic demonstrating

that the protein is non-allergenic [65], the GRAVY hydropathicity with negative value(-0.219) indicates that the protein is hydrophilic , other characters point to be stable with aliphatic nature The 3D structure of the protein was estimated using Phyer2.0 server , visualized using PyMOL as shown in Fig 4

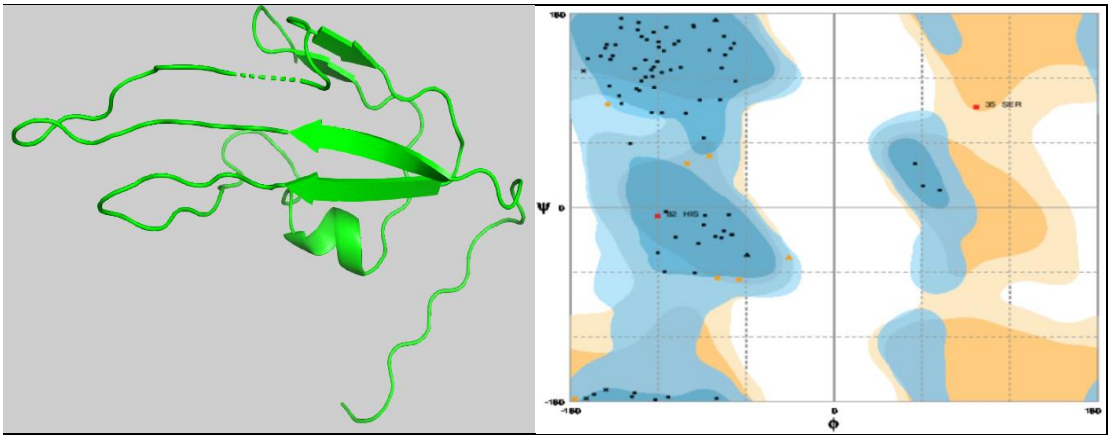
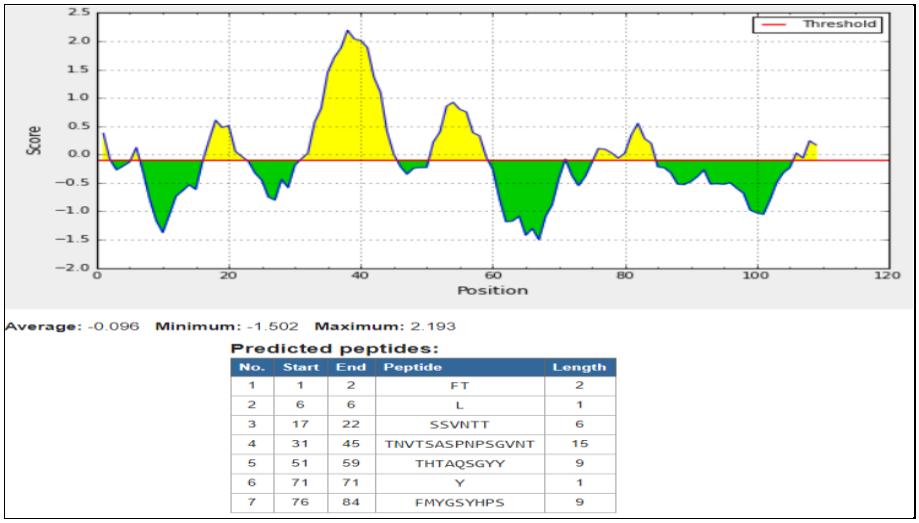


Fig 4: 3D structure of selected protein **Fig 5: Ramachandran plot of protein**

The protein rich in loops and turns, which are highly changeable structures in protein [66]. The distribution and validity of amino acids according to Ramachandran plot as in Fig 5. The number of residues in favored region 90% and residues in allowed region 7.8%, while the outliner region represents only 2.2%. These efforts were done according to the fact that global genotypes or strains can be considered for development of novel multivalent universal vaccines [1], and a regional vaccination strategy based on specific local strains can be adopted in addition to the general vaccines based on ubiquitous genotypes .

Designing of vaccine epitopes : Effective vaccine should be able to elicit both humoral and cell mediated immunity responses which is essential to complete eradication the chance of reinfection [27] . This vaccine could be predicted in accordance with the genotype of field isolates. And since the S1 glycoprotein is responsible for infection of host cells, consequently the S1 protein is usually used for IBV typing [67] . Combination of antigenic epitopes of B cells and T cells that are conserved across many strains as a approach to evoke humoral and cytotoxic T cell (CTI) immune responses will potentially lead to a broad –based vaccine that could reduce the challenges in using live attenuated vaccine technology in the control of IBV infection in poultry .

B cell epitope prediction : Linear B cell epitopes located in the S1 region have been reported to play a role in virus neutralization [68] . BepiPred /IEDB tool was used to predict linear/continuous B cell epitopes , this type of epitope prediction is mainly based on the amino acids properties such as hydrophilicity , charges ,exposure to the surface area and secondary structure [27] , using default setting of the tool resulted in many epitopes as shown in Fig 6



No.	Start	End	Peptide	Length
1	1	2	FT	2
2	6	6	L	1
3	17	22	SSVNTT	6
4	31	45	TNVTSASPNPSGVNT	15
5	51	59	THTAQSGYY	9
6	71	71	Y	1
7	76	84	FMYGSYHPS	9

Fig 6 : Predicted B cell epitopes using BepiPred / IEDB

The hydrophilicity which is an important character of B epitope was estimated as in Fig 7

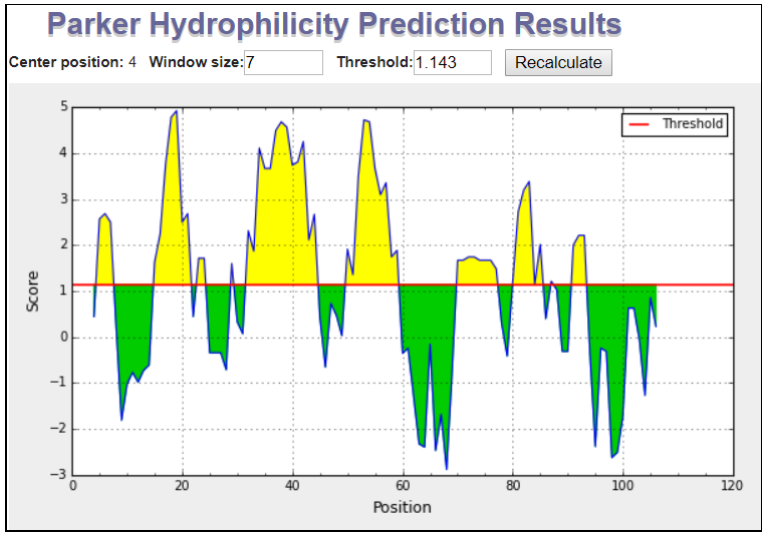


Fig 7 : The hydrophilicity of B cell epitopes

In addition other characters were estimated such as flexibility , accessibility and Beta turn prediction as shown in Fig 8A, 8B, 8C

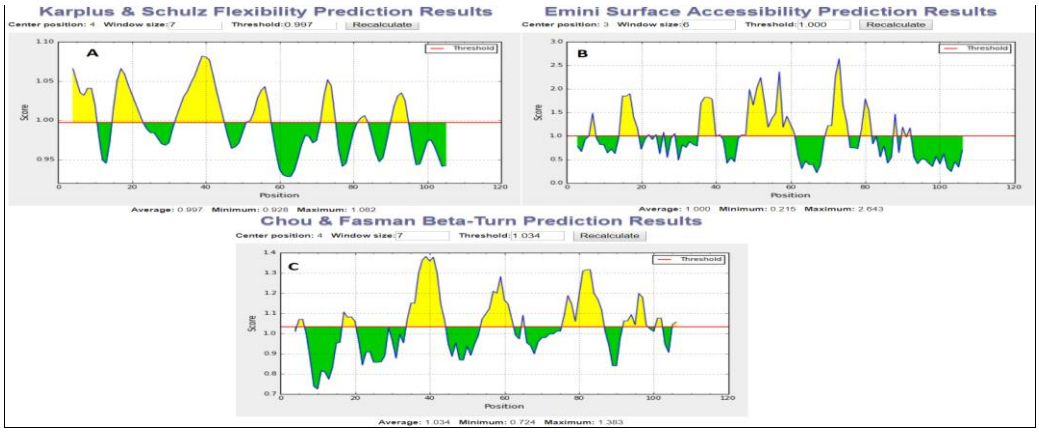


Fig 8 : Some characters of B cell epitopes

All these epitopes were filtered according to their antigenic score and surface position using THMMH software, only two epitopes satisfied these criteria ; 51-59(THTAQSGYY) and 76-84(FMYGSYHPS) the former with antigenic score 0.4794 ,and the second one 0.5662, those were subjected for further studies . These epitopes showed a good existence and distribution among the test strains (Iraqi and variant 2 strains) (Fig 9)

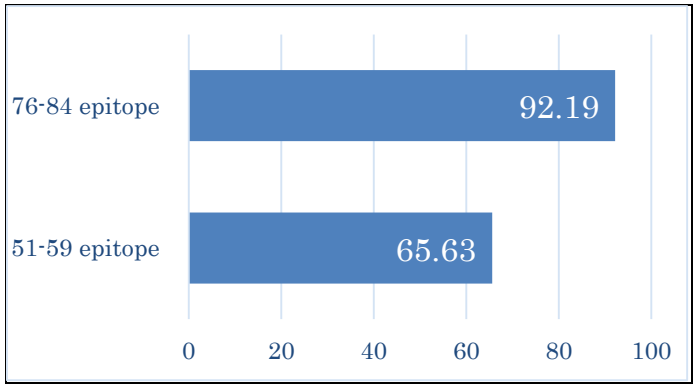


Fig 9 : Distribution of selected (predicted) B cell epitopes among Iraqi and variant 2 Strains (Test strains)

Cytotoxic T cell epitopes prediction: Broad CTL response against IBV is one of the crucial factors that help to control viral replication , spike protein on the surface of IBV viron harbor major T cell epitopes [52] (8) . NetMHCpan version 4.0was used to predict the CTL epitopes with 9mer and percentile rank value less than 1, this prediction gives the MHC I alleles interact with each epitope , according to the results of the server only strong binders (SB) were chosen as the affinity less than 50nM for several alleles, since these epitopes tend to be potential candidates for epitope-based vaccine design [30,48] . Several epitopes (9mer) were obtained, these were subjected to estimation of antigenic score using VaxiJen v2.0 and surface position , in addition to estimation of TAP score [44] , only 5 epitopes were satisfied the parameters , these are shown in Table 2

Table 2 : Predicted CTL epitopes , their position in selected protein, antigenic score and representative alleles they react with

Epitope, position and sequence	Antigenic score	Alleles*
22-30 TLELTNFTF	2.2281	HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*26:01, HLA-B*07:02, HLA-B*08:01, HLA-B*27:05, HLA-B*39:01, HLA-B*40:01, HLA-B*58:01, HLA-B*15:01,
57-65 GYYNFNFSF	2.5757	HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*26:01,HLA-B*07:02, HLA-B*08:01, HLA-B*27:05, HLA-B*39:0,HLA-B*40:01, HLA-B*58:01, HLA-B*15:01

68-76 SFVYKESNF	1.4594	HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*26:01, HLA-B*07:02, HLA-B*08:01, HLA-B*27:05, HLA-B*39:01, HLA-B*40:01, HLA-B*58:01, HLA-B*15:01
73-81 ESNFMYGSY	0.9007	HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*26:01, HLA-B*07:02, HLA-B*08:01, HLA-B*27:05, HLA-B*39:01, HLA-B*40:01, HLA-B*58:01, HLA-B*15:01
99-107 NSLSVSLAY	1.3095	HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*26:01, HLA-B*07:02, HLA-B*08:01, HLA-B*27:05, HLA-B*39:01, HLA-B*40:01, HLA-B*58:01, HLA-B*15:01

*Only representative alleles were used

These epitopes were checked for their existence in S1 protein of test strains , Fig 10 shows the percent of the existence at two level of similarity , 100% and 90%

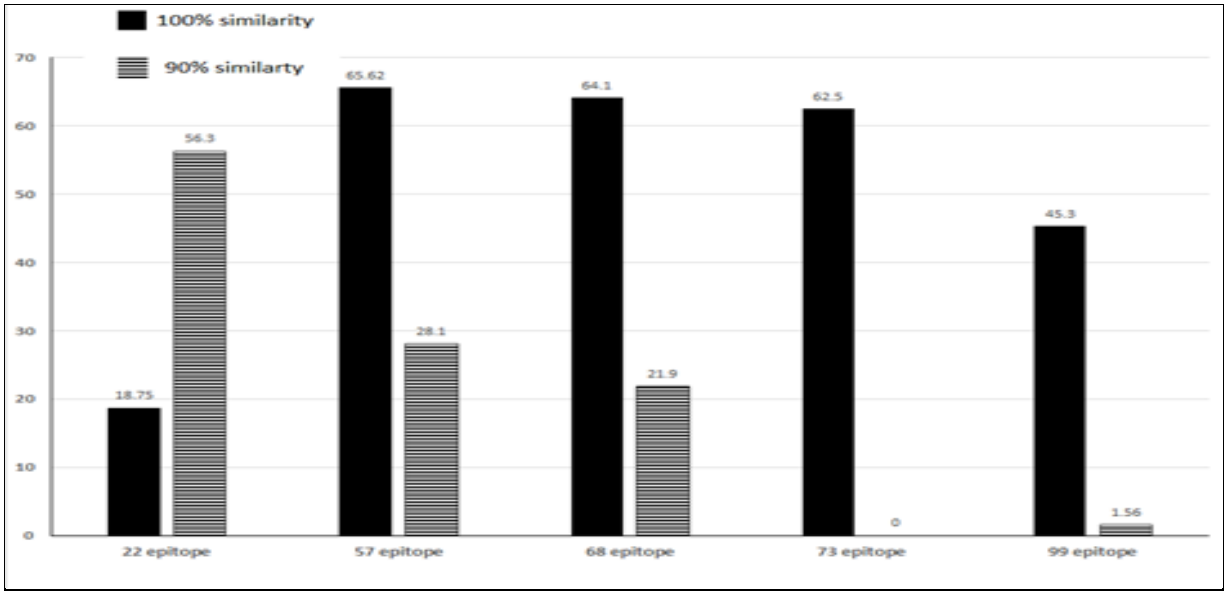
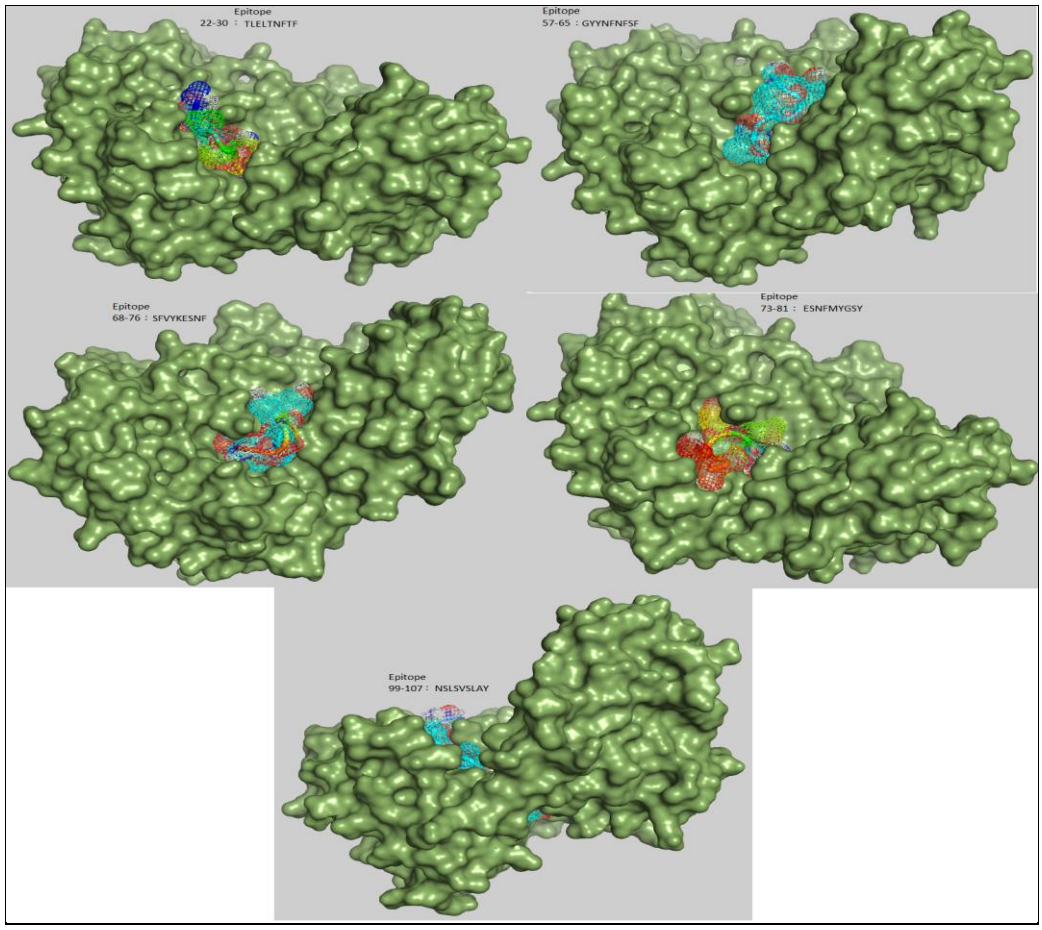


Fig 10 : Existence and distribution of CTL cell epitopes among test strains

It is known that CTLs play a crucial role in fighting viral infections after interaction with MHC I alleles [69]. MHC I alleles (HLA-A,-B) were chosen instead of B-F alleles (MHC I alleles of chickens) due to non-availability of B-F alleles by software and due to similarity between human MHC I and B-F biochemically and functionally in antigen presentation and their stimulation of immune system [69]. In addition the anchor residues in chicken BF2 haplotype are similar to residues anchored on mammalian MHC I, typically 8- , 9mer in size [52], so since a little information is available on the biological function of those molecules in poultry [70], HLA alleles are used during computational stage of prediction of epitopes, and then used the resulted epitopes for docking on the available chicken allele, The epitopes can interact with many representative HLA alleles as shown in Table 2. These epitopes were converted in 3D structure using PEP-FOLD 3.0 and used for

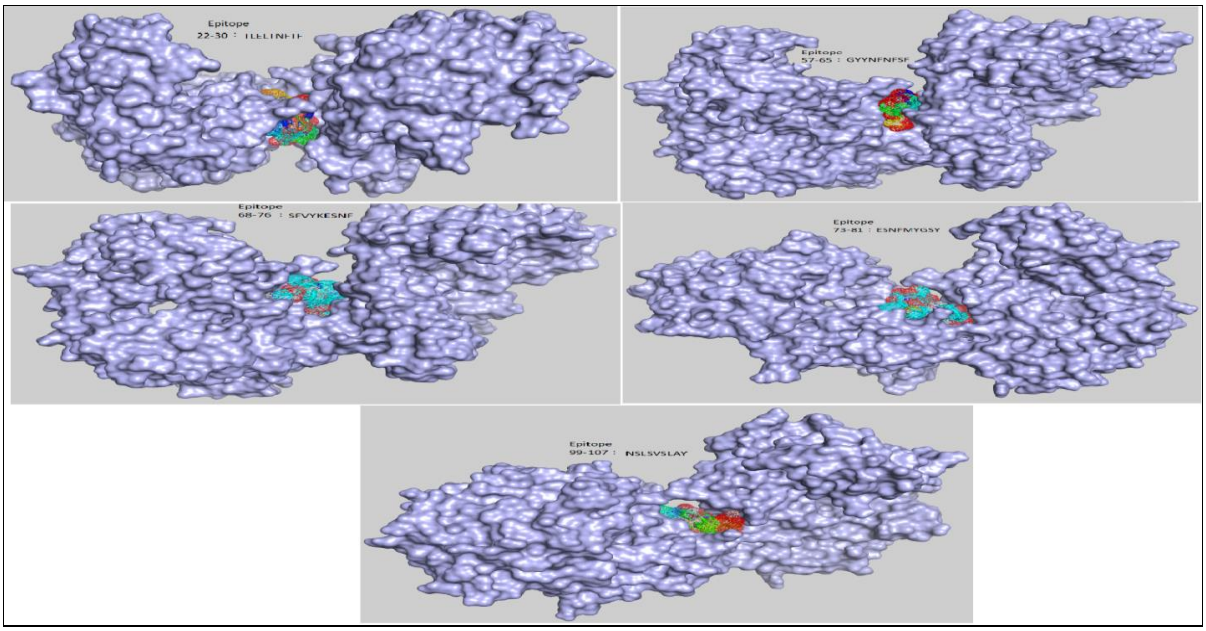
docking using PyRx software version 8.0 with the chicken MHC I molecule BF2*201(pdb ID 3ebv) and BF2*0401 (pdb ID 4g42) . The allele BF2*201 as suggested by Kokh et al., 2007[71] as a promiscuous peptide binding and is one of the most expressed MHC I alleles in chickens with an uniqueness flexibility of binding different peptides. The docking results of designed CTL epitopes with BF2*201 are shown in Fig 11 which is visualized by PyMOL software



Epitope position and sequence	ΔG (kcal/mol)	RMSD
22-30: TLELTNFTF	-8.0	0
57-65: GYYNFNFSF	-9	0
68-76: SFVYKESNF	-8.7	0
73-81 :ESNFMYGSY	-9.6	0
99-107 :NSLSVSLAY	-8.5	0

Fig 11: Docking of CTL epitopes with BF2*201 chicken MHC I molecule

The parameters for docking used were the free energy changes (ΔG) and RMSD values [72,73]. Docking of CTLs epitopes with other available allele BF2*0401 is shown on Fig 12



Epitope position and sequence	ΔG (kcal/mol)	RMSD
22-30:TLELTNFTF	-7.6	0
57-65:GYYNFNFSF	-8.0	0
68-76:SFVYKESNF	-7.8	0
73-81:ESNFMYGSY	-7.5	0
99-107:NSLSVSLAY	-7.4	0

Fig 12: Docking of CTL epitopes with BF2*0401 chicken MHC I molecule

These CLTs epitopes docked and interact with BF2*201 and BF2*0401 suggests the existence of real CTL epitopes [70] .

Helper T cell epitope prediction: Helper T cell required to activation of B cells and other immune system cells , these epitopes interact with MHC II molecules (HLA-DR,-DP,-DQ) , and due difficulty to determine avian B-L alleles [69], and serological , structural and functional studies of B-L antigens showed that a great similarity with 1a antigen of mammals [63], the later was used for prediction of helper T cell epitopes using NetMHCIIpan 3.2 [45] to estimate the epitope for 43

strains . EpiTop 1.0 was used to estimate the epitopes of the rest tested strains using all binder [46] , as the basic approaches of the two software are different. Epitopes predicted with NetMHCIIpan 3.2 were found in 95.3-100% of the strains with different alleles indicate in Table 3A , the results showed the alleles and the affinity (nM)

Table 3A : Helper T cell epitopes predicted by NetMHCIIpan 3.2 showing the alleles they react with them and the affinity (nM)

Sequence position	Alleles	Affinity nM
56-70 SGYYNFNFSFLSSFV	DRB1_1526, DRB1_1527, DRB1_1529, DRB1_1530, DRB1_1531, DRB1_1534, DRB1_1535, DRB1_1538 DRB1_1539, DRB1_1544, DRB1_1547,DRB1_0469, DRB1_1502, DRB1_1511, DRB1_1514, DRB1_1515, DRB1_1519,	101.18, 75.22, 32.07, 51.9, 80.9, 38.48, 36.08, 75.22, 75.22, 75.22, 75.22, 276.68, 75.22, 74.73, 75.22, 61.85, 75.22,
	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10107-DPB10401, HLA-DPA10108-DPB10402, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10303-DPB110001,	134.82, 28.00, 39.73, 42.09, 109.49, 80.65, 141.22, 168.48, 137.16,
57-71 GYYNFNFSFLSSFVY	DRB1_0701, DRB1_0703, DRB1_0705, DRB1_0706, DRB1_0707, DRB1_0708, DRB1_1523, DRB1_1526, DRB1_1527, DRB1_1529, DRB1_1530, DRB1_1531, DRB1_1534, DRB1_1538, DRB1_1539, DRB1_0713, DRB1_0714, DRB1_0715, DRB1_0716, DRB1_0717, DRB1_0719, DRB1_1544, DRB1_1547, DRB1_1602, DRB1_1605, DRB1_1607, DRB1_1611, DRB1_0901, DRB1_0907, DRB1_0908, DRB1_0909, DRB1_0424, DRB1_1614, DRB1_1616, DRB1_0469, DRB1_1502, DRB1_1503, DRB1_1511, DRB1_1514, DRB1_1515, DRB1_1519,	38.11, 39.85, 39.85, 68.81, 39.85, 39.85, 84.80, 71.47, 25.85, 51.90, 80.90, 35.40, 30.30, 71.47, 71.47, 39.85, 39.85, 39.85, 39.85, 39.85, 39.85, 71.47, 71.47, 63.55, 106.09, 106.09, 63.55, 56.69, 54.16, 153.54, 56.69, 117.29, 72.22, 63.55, 242.87, 71.47, 130.97, 71.92, 71.47, 49.98, 71.47,

	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10107-DPB10401, HLA-DPA10108-DPB10402, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10303-DPB110001,	117.58, 25.44, 34.71, 36.43, 93.84, 69.66, 123.63, 139.71, 111.15, 139.71, 111.15, 118.71,
58-72 YYNFNFSFLSSFVYK	DRB1_0480, DRB1_0483, DRB1_0484, DRB1_0489, DRB1_0701, DRB1_0703, DRB1_0705, DRB1_0706, DRB1_0707, DRB1_0708, DRB1_0709, DRB1_0711, DRB1_0712, DRB1_0405, DRB1_0445, DRB1_0448, DRB1_0457, DRB1_0114, DRB1_1523, DRB1_1526, DRB1_1527, DRB1_1529, DRB1_1530, DRB1_1531, DRB1_1534, DRB1_1538, DRB1_1539و DRB1_0713, DRB1_0714, DRB1_0715, DRB1_0716, DRB1_0717, DRB1_0719, DRB1_1544, DRB1_1548, DRB1_1602, DRB1_1604, DRB1_1605, DRB1_1607, DRB1_1609, DRB1_1610, DRB1_1611, DRB1_1612, DRB1_0901, DRB1_0905, DRB1_0907, DRB1_0908, DRB1_0909, DRB1_1001, DRB1_1003, DRB1_0424, DRB1_0429, DRB1_0430, DRB1_1614, DRB1_1616, DRB1_0462, DRB1_0469, DRB1_0477, DRB1_1502, DRB1_1503, DRB1_1507, DRB1_1511, DRB1_1514, DRB1_1515, DRB1_1519,	77.14, 91.53, 91.53, 91.53, 35.30, 35.30, 35.30, 58.31, 35.30, 35.30, 46.86, 36.98, 32.94, 91.53, 91.53, 91.53, 91.53, 22.00, 61.36, 76.25, 23.60, 46.13, 84.72, 35.07, 27.45, 76.25, 76.25و35.30, 35.30, 35.30, 35.30, 35.30, 35.30, 76.25, 75.28, 49.69, 92.55, 86.19, 86.19, 80.21, 41.41, 49.69, 52.46, 50.39, 57.73, 49.17, 118.97, 50.39, 23.23, 23.23, 103.02, 91.53, 91.53, 56.65, 49.69, 75.64, 249.98, 91.53, 76.25, 96.10, 75.28, 77.09, 76.25, 40.60, 76.25,
	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10107-DPB10401, HLA-DPA10108-DPB10402, HLA-DPA10110-DPB10601, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10303-DPB110001,	97.45, 21.56, 28.61, 30.18, 75.13, 288.94, 56.52, 96.99, 110.57, 85.27, 93.53

61-75 FNFSFLSSFVYKESN	DRB1_0480, DRB1_0483, DRB1_0484, DRB1_0487, DRB1_0489, DRB1_0706, DRB1_0405, DRB1_0409, DRB1_0445, DRB1_0448, DRB1_0457, DRB1_1523, DRB1_1534, DRB1_1548, DRB1_1601, DRB1_1602, DRB1_1603, DRB1_1604, DRB1_1608, DRB1_1609, DRB1_1611, DRB1_0908, DRB1_1001, DRB1_1003, DRB1_0417, DRB1_0424, DRB1_0428, DRB1_0429, DRB1_0430, DRB1_1614, DRB1_1616, DRB1_0462, DRB1_0469, DRB1_0477, DRB1_1503, DRB1_1507, DRB1_1512, DRB1_1515,	66.74, 76.18, 76.18, 73.95, 76.18, 67.23, 76.18, 96.37, 76.18, 76.18, 76.18, 67.69, 37.49, 87.63, 89.23, 62.30, 89.23, 86.51, 89.23, 70.79, 62.30, 90.95, 20.97, 20.97, 274.56, 274.56, 85.82, 67.29, 76.18, 76.18, 64.99, 62.30, 70.79, 256.32, 76.18, 119.10, 87.63, 98.40, 44.27,
	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10106-DPB10301, HLA-DPA10107-DPB10401, HLA-DPA10108-DPB10402, HLA-DPA10109-DPB10501, HLA-DPA10110-DPB10601, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10303-DPB110001,	74.58, 24.11, 28.80, 354.00, 31.84, 68.41, 199.23, 224.22, 49.63, 79.59, 80.94, 71.28, 71.99,
63-77 FSFLSSFVYKESNFM	DRB1_0480, DRB1_0409, DRB1_0908, DRB1_0424,	86.97, 107.68, 107.47, 124.83,
	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10106-DPB10301, HLA-DPA10107-DPB10401, HLA-DPA10108-DPB10402, HLA-DPA10109-DPB10501, HLA-DPA10110-DPB10601, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10303-DPB110001,	82.76, 29.50, 35.12, 400.12, 37.90, 81.12, 212.87, 270.90, 53.37, 91.11, 92.94, 74.85, 78.65,
64-78 SFLSSFVYKESNFMY	DRB1_0409, DRB1_0908, DRB1_0462, DRB1_1511, DRB1_1515,	110.22, 142.04, 80.14, 131.59, 63.08,
	HLA-DPA10103-DPB10101, HLA-DPA10104-DPB10201, HLA-DPA10105-DPB10202, HLA-DPA10107-DPB10401, HLA-	101.08, 36.56, 42.53, 50.87, 107.82,

	DPA10108-DPB10402, HLA-DPA10109-DPB10501, HLA-DPA10201-DPB10801, HLA-DPA10203-DPB110001, HLA-DPA10204-DPB10601, HLA-DPA10301-DPB10801, HLA-DPA10303-DPB110001,	267.69, 67.41, 118.93, 142.21, 82.04, 95.10,

While Table 3B shows the helper epitopes predicted for the second group of the tested strains using EpiTop1.0 (see above)

Table 3B : Helper T cell epitopes of some strains predicted by EpiTop 1.0

Test strains	Position	Sequence	log(1/IC50)
ACT64163.1 IRAQ 2	97 98 101 96	WFNSLSVSL FNSLSVSLA LSVSLAYGP LWFNSLSVS	5.740
ALQ11066.1 IRAQ 3	92	INNGLWFNS	5.740
ALQ11065.1 IRAQ 4	23 11	LELTNFTFT FIVYRESSV	5.740
ALQ11063.1 IRAQ 6	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11062.1 IRAQ 7	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11061.1 IRAQ 8	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11060.1 IRAQ 9	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11059.1 IRAQ 10	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11058.1 IRAQ 11	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11057.1 IRAQ 12	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11056.1 IRAQ 13	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11054.1 IRAQ 14	92 23	INNGLWFNS LELTNFTFT	5.740
ALQ11053.1 IRAQ 15	92	INNGLWFNS	5.740

ALQ11052.1 IRAQ 16	76 25	FMYGSYHPS LTNFTFTNV	5.740
ALQ11051.1 IRAQ 17	76 77	FMYGSYHPS MYGSYHPSC	5.740
ALQ11050.1 IRAQ 18	76 77	FMYGSYHPS MYGSYHPSC	5.740
ABK63949.1 IRAQ 37	76 77	FMYGSYHPS MYGSYHPSC	5.740

In any case, future IBV vaccine must induce broad protection against different IBV serotypes and to meet the international safety regulations and be easier to apply and cost effective for wider acceptance which is significant advantages over inactivated and live attenuated vaccines in inducing efficient antigenic presentation, high stability , flexibility in epitope selection by poultry industry and is necessary and favored [52,64]. Synthetic peptide/epitope vaccines have been shown able to meet such requirements and allow induction and optimization of the desired type of immunity [9,64], and there is no infection with viral particles [64] and no mutations and away from recombination processes. Some researchers have focused on developing multi-epitope vaccines (as in this study) to be used against wide range of IBV serotypes. The use of such vaccines will likely reduce the challenges associated with live attenuated vaccines and allow broad coverage of the target IBV strains and offers broad and specific responses in single administration [64,68] .

4. CONCLUSION:

In conclusion, in this study different epitopes were predicted and can be used in combination depending on the case especially for variant 2 which is not responding to available vaccines and considered as a disaster in Iraq.

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6. CONFLICT OF INTEREST:

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