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Research Article

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Computational Analysis for Prediction of Multi Epitopes Vaccine against Blue Tongue Virus Serotype 4 from VP5 and VP7 Proteins

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Abstract

Blue Tongue Disease (BTD) is a non-contagious insect transmitted disease of ruminants caused by double stranded RNA virus. This study aimed to predict an effective multi-epitopes vaccine against BTD from VP5 and VP7 as immunogenic proteins using immunoinformatic tools. The VP5 and VP7 proteins sequences were retrieved from GenBank of National Center for Biotechnology Information (NCBI). The sequences of each protein were aligned for conservancy using Bioedit software. Immune Epitope Database (IEDB) analysis resources were used to predict B and T cell epitopes. The proposed MHC-1 epitopes of both proteins were further subjected to molecular docking to show minimum binding energy of each epitopes. In our results, two epitopes (235-SEEV-235 and 85-PDPLSP-90) from VP5 and two epitopes (79-PISPDYTQ-86 and 297-PIFPPN-302) from VP7 were proposed as B cell epitopes since they were shown to be linear, surface accessible and antigenic epitopes. For T cells, MHC-1 binding prediction tools showed multiple epitopes strongly interacted with BoLA alleles from both VP5 and VP7. Among them three epitopes, (257-KLKKVINAL-265, 487-QMHILRGPL-495 and 350-VMMRFKIPR-358) fromVP5 protein and four epitopes (86-QHMATIGVL-94, 315-TLADVYTVL-323, 17-TLQEARIVL-25 and 10-TVMRACATL-18) from VP7 protein interacted with the highest number of alleles and demonstrated best binding affinity to MHC-1 alleles. Thus were proposed as a vaccine candidate from VP5 and VP7 proteins. All the epitopes from VP5 and VP7 that interacted with MHC-1 alleles when subjected to molecular docking against the sheep b_microglobulin alleles demonstrated biologically significant higher binding affinity which expressed by their lower global and attractive energy. In conclusion, eleven epitopes were predicted as promising vaccine candidates against BTD from the VP5 and VP7 immunogenic proteins. These epitopes require to be validated experimentally through in vitro and in vivo studies.

Keywords: BTD, multi epitope vaccine, IEDB, NCBI, B-lymphocytes, T lymphocyres

Introduction

The blue tongue disease (BTD) is a non-contagious insect transmitted disease of ruminants, particularly sheep and certain species of non-African wild ruminant [1, 2]. The disease is caused by blue tongue virus (BTV). The virus is double stranded RNA virus belongs to the genus orbivirus in the family reoviridae [3, 4]. The orbiviruses genome encodes for four nonstructural protein (NS1-NS4) and seven structural proteins (VP1-VP7) [3, 4]. The genome is segmented and composed of ten segments packaged with an icosahedral capsid, ~80 nm in diameter, and three concentric protein layers [5]. The outer core composed of 60 trimers of VP2 and 120 of trimers of VP5. The mid-layer contained the virus genome, the viral transcriptase complexes and provided a 'scaffold for addition of 780 copies of VP7 (organized as 260 trimers) to form the core-surface layer. In the inner most layer the 'sub core shell was found and constructed from 12 decamers of VP3 [5]. When the Merino sheep were imported in to South Africa became infected with The BTV disease since 1900 [6]. The disease transmitted via several species of biting midges belonging to the genus Culicoides by different way, vector-free transmission, and vertical from dam to fetus [7-9]. Also, horizontal transmission via direct contact of BTV-26 isolated for the first time from symptomatic sheep in Kuwait in 2010 was reported [10]. In this direct contact transmission, the virus does not replicate in midges and Culicoides-derived KC cells [11, 12].

The clinical symptoms of the disease is characterized by vascular injury that resulted in hemorrhage and ulceration of the mucous membranes in the upper portion of the gastrointestinal tract; coronitis and laminitis; facial and inter muscular oedema; pleural and pericardial effusion; pulmonary oedema and necrosis of skeletal and cardiac muscle [13-15]. Moreover, the symptoms of the BTV

serotype-8 caused reproductive failure among pregnant cattle and sheep. Also, serotype-8 infection has high rate of vertical virus transmission that lead to teratogenic defects in congenitally infected calves [16-18]. There is extensive heterogeneity of the field strains of BTV. This variations lead to different virulence and other biological properties of individual field strains of the virus. This variation result from genetic shift and genetic drift. The later was as a result of re-assortment of viral genes during mixed infections of either the vertebrate or invertebrate hosts following infection with more than one virus serotype or strain [19].

The BTV has more than one immunogenic protein. TheVP2 is the primary target of neutralizing antibody responses but high variability permits differentiation of the 26 BTV serotypes due to this known as serotype specific neutralizing antibody induction [20-24]. While it has been suggested that vp5 may aid induction supporting vp2 tertiary conformation [22]. VP7 which high immunogenic and widely used in serological diagnosis, some inner capsid proteins may induce various degrees of immunity; their specific contributions to protection are not fully elucidated [25-28]. Also, some nonstructural proteins have a role in immunity by produce protective cell-mediated immune responses [29, 30]. These non-structure proteins are NS1 and NS2 which induce specific humoral or cellular immunity, while NS3 is understood to induce specific immunological responses but to a lesser degree [31-34].

Various types of vaccines were uses to prevent BTV infection since 1908 [35]. Modified live vaccine was used to control the disease in sheep in southern Africa, and more recently in Corsica, the Balearic Islands and Italy [36]. Also live attenuated vaccine was used and demonstrated effective protection. However, animals showed clinical signs of bluetongue infection; teratogenicity and adverse effects were also reported [37]. Whole inactivated vaccines, which represent safer than live attenuated vaccine and are commercially available. However, some concerns exist over the reliability of inactivation for each vaccine batch [38]. The recombinant vaccine was also used against BTV but constraints imposed by the high cost of performing experiments in bio-containment facilities for large animals have led to the establishment of a small animal model for BTV [39]. DNA vaccines that characterized by safety, easy manufacturing, biological stability and cost effectiveness but have low immunogenicity. They can be useful to prime the immune system when used in heterologous vaccination regimes in combination with recombinant viruses as boosting agents. This heterologous vaccination strategy was successfully used in various studies [40-42].

In silico vaccines tools for vaccine design was developed since 1980s to predict T and B cell immune epitope [43]. This method used computational and experimental tools to identify the immunogenic part or antigen suited to form vaccine prediction. Sometimes the intact protein is used for prediction of the vaccine or corresponding RNA or DNA [44-47]. In silico, vaccines are simple in production in a well-controlled process. Also in silico, vaccines can be designed to provoke an immune response that is very specifically directed to highly immunogenic regions of antigens [48, 49]. This vaccine design process tailored to specific MHC alleles and combinations thereof provides the basis of safer personalized therapy [48, 49]. Thus, the need for a safer and efficacious vaccine without future complications is highly recommended. In this study, we aimed to use the immunoinformatics approaches found in the Immune Epitope Database (IEDB) to predict epitopes from VP5 andVP7 proteins of BTV that elicit the immune system and acted as safer efficacious vaccine.

Materials and Methods

Protein sequences retrieval and alignment tool

The protein sequences of VP5 (56 sequences) and VP7 (65 sequences) were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/protein/?term=bluetongue+vp5,vp7) [50]. The protein strains were retrieved according to their accession numbers, country and date of collection and were presented in table (1).

Table 1: The retrieved strains with accession numbers, countries and year of collection (vp5) and (vp7)

VP5			VP7		
Accession	Country	Year	accession	Country	Year
*YP-052955.1	USA	2004	**YP_052967.1	USA, England	2004;1988
AKV61130.1	Sudan	1983	AFV67788.1	China	2012
AKV61129.1	Spain	2010	AAW32489.1	South Africa	1979-2004
AKV61128.1	Spain	2005	AAW32488.1	South Africa, Greece	1979-2004;1999
AKV61127.1	Spain	2004	AAW32487.1	South Africa, Greece	1979-2004;2000
AKV61126.1	Spain	2003	AAW32483.1	South Africa, France	1979-2004;2003
AKV61125.1	Cyprus	1969	AKH40942.1	Hungary	2014
AKV61124.1	Morocco	2009	AIS39803.1	Spain	2003
AKV61123.1	Morocco	2009	ABG91359.1	South Africa, Turkey	1979-2004;1999
AKV61122.1	Morocco	2009	AQW44904.1	France	2016
AKV61121.1	Morocco	2009	AYA21811.1	South Africa	2017
AKV61120.1	Morocco	2004	AYA21810.1	South Africa	2017
AKV61119.1	Israel	2008	AYA21809.1	South Africa	2017
AKV61118.1	Israel	2006	AYA21807.1	South Africa	2017
AKV61117.1	Greece	2012	AYA21804.1	South Africa	2017

AKV61116.1	Greece	2012	AYA21796.1	South Africa	2017
AKV61115.1	Greece	2012	AYA21795.1	South Africa	2017
AKV61114.1	Greece	2000	AYA21792.1	South Africa	2016
AKV61113.1	Greece	2000	AXQ59259.1	France	2017
AKV61112.1	Greece	1999	AKV61252.1	Sudan	1983
AKV61111.1	Greece	1999	AKV61251.1	Spain	2010
AKV61110.1	Greece	1979	AKV61249.1	Spain	2004
AKV61109.1	France	2003	AKV61250.1	Spain	2005
AKV61108.1	France	2003	AKV61248.1	Spain	2003
AKV61107.1	France	2003	AKV61247.1	Cyprus	1969
AKV61106.1	Egypt	1977	AKV61246.1	Morocco	2009
AKV61105.1	Cyprus	2011	AKV61245.1	Morocco	2009
AKV61104.1	Cyprus	2011	AKV61244.1	Morocco	2009
AKV61103.1	Cyprus	2011	AKV61243.1	Morocco	2009
AKV61102.1	Cyprus	2011	AKV61242.1	Morocco	2004
AKV61101.1	Cyprus	2011	AKV61241.1	Israel	2008
AKV61100.1	Cyprus	2004	AKV61240.1	Israel	2006
AKV61099.1	Cyprus	1969	AKV61239.1	Greece	2012
AIL52757.1	South Africa	N/A	AKV61238.1	Greece	2012
AEO19755.2	South Africa	N/A	AKV61237.1	Greece	2012
AGJ83446.1	South Africa	N/A	AKV61236.1	Greece	2000
AFV67787.1	China	1997	AKV61235.1	Greece	2000
AEO19827.1	Italy	2003	AKV61234.1	Greece	1999
AEO19815.1	Italy	2003	AKV61233.1	Mandriko	1999
CAE53015.1	South Africa	N/A	AKV61232.1	Greece	N/A
CAE52998.1	Argentina	N/A	AKV61231.1	France	2003
CAE52997.1	Greece	N/A	AKV61230.1	France	2003
CAE52996.1	Turkey	N/A	AKV61229.1	France	2003
CAE52995.1	Turkey	N/A	AKV61228.1	Egypt	1977
CAE52994.1	Cyprus	N/A	AKV61227.1	Cyprus	2011
CAE52993.1	Sudan	N/A	AKV61226.1	Cyprus	2011
CAE52992.1	South Africa	N/A	AKV61225.1	Cyprus	2011
AIK27564.1	India	2008	AKV61224.1	Cyprus	2011
CAH04360.1	Spain	N/A	AKV61223.1	Cyprus	2011
CAH04359.1	Spain	N/A	AKV61222.1	Cyprus	2004
ALU65919.1	South Africa	2011	AKV61221.1	Cyprus	1969
AFX79650.1	Argentina	2010	AIL52758.1	South Africa	2014
AFX79644.1	Argentina	2009	AEO19754.1	South Africa	2011
AFX79638.1	Argentina	1999	AGJ83447.1	N/A	2012
AFX79632.1	Argentina	1999	AEO19826.1	Italy	2003
AFX79626.1	Argentina	1999	AEO19814.1	Italy	2003
			ABO34077.1	Portugal	2004
			AAF97691.1	China	N/A
			AIK27565.1	India	2008
			ALU65921.1	South Africa	2011
			AFX79651.1	Argentina	2010

			AFX79645.1	Argentina	2009
			AFX79639.1	Argentina	1999
			AFX79633.1	Argentina	1999
			AFX79627.1	Argentina	1999

*Reference sequence of vp5

**Reference sequence of vp7

N/A: not available

Molecular evolution analysis

The retrieved sequences of VP5 and VP7 were subjected to evolutionary divergence analysis and a phylogenetic tree was constructed to determine the common ancestor of each strain using MEGA 7.0.26 (7170509-x86_64) [51].

Multiple sequence alignment

The protein sequences of the retrieved strains of VP5 and VP7 were further aligned to obtain the conserved regions using multiple sequence alignment (MSA) tools of the Clustal W in the Bio Edit program, version 7.0.9.0 [52].

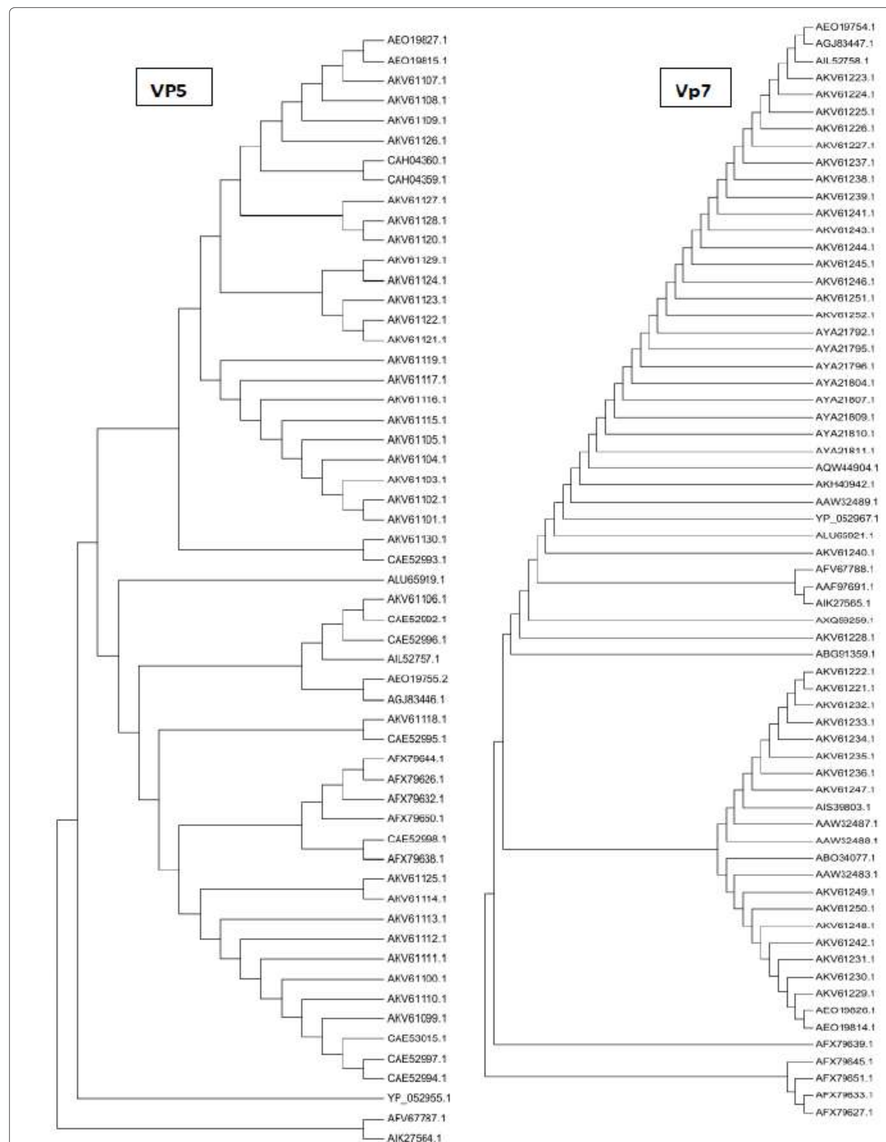

Figure 1: Phylogenetic tree of VP5 and VP7. The retrieved strains demonstrated divergence in their common ancestors

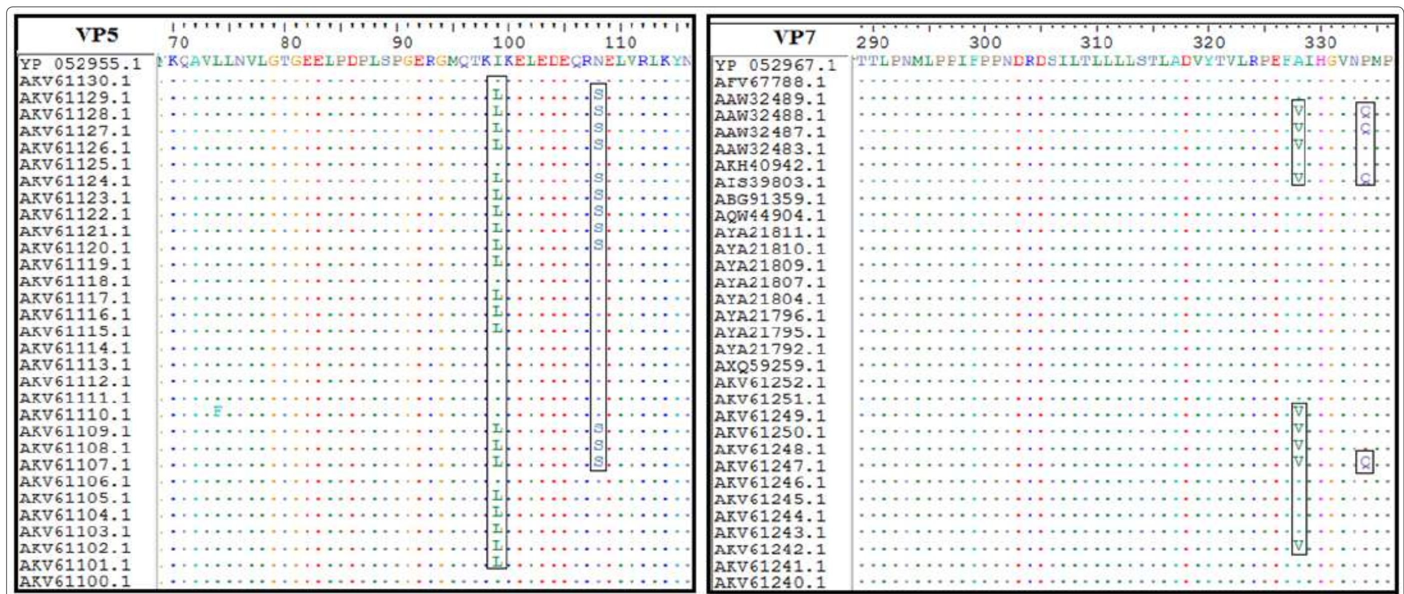

Figure 2: Multiple sequence alignment showed the conservancy between sequences of the retrieved strains of VP5 and VP7 proteins. The alignment was performed using BioEdit software tool. Dots showed the conserved regions while rectangular within the sequences showed the mutated or the unconserved region between strains

B-lymphocytes epitopes prediction

Tools from IEDB (http://tools.iedb.org/bcell/), were used to identify the B cell epitopes, including Bepipred for linear epitope analysis with a default threshold value of 0.350 for both VP5 and VP7, Emini for surface accessibility with the default threshold value 1.000 for both VP5 and VP7 and Kolaskar and Tongaonkar for antigenicity scale with a default threshold values of 1.019 and 1.026 for VP5 and VP7 respectively [54-56].

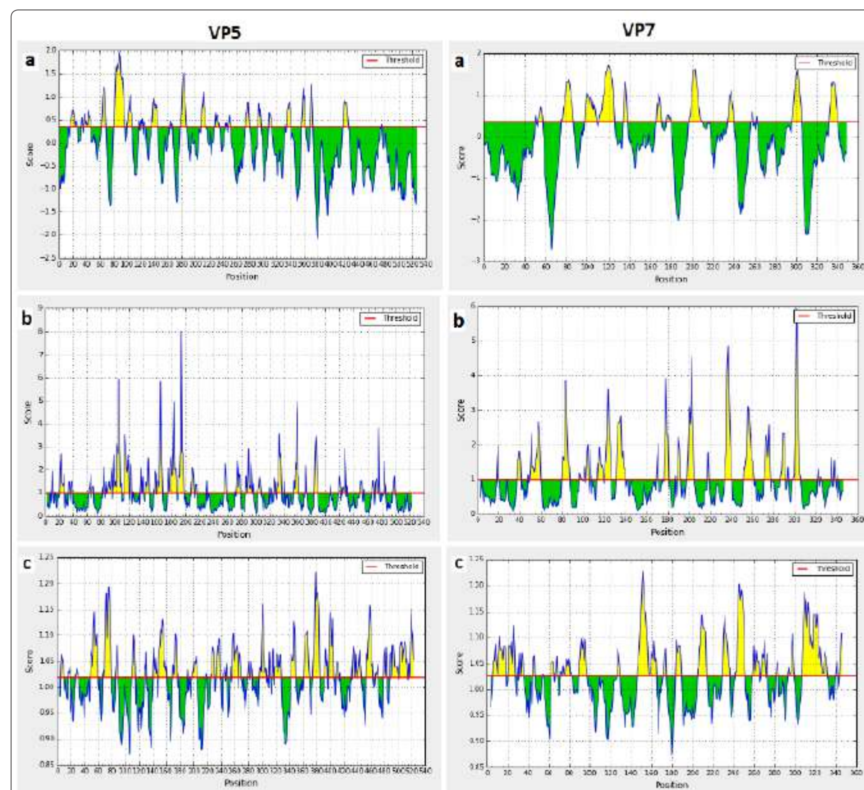

Figure 3: Prediction of B-cell epitopes of VP5and VP7 by different IEDB scales (a- Bepipred linear epitope prediction, b- Emini surface accessibility, c- Kolaskar and Tongaonkar antigenicity prediction). Regions above threshold (red line) are proposed to be a part of B cell epitope while regions below the threshold (red line) are not

T-lymphocytes epitopes prediction

The IEDB was used for the identification of the T cell epitopes prediction. The prediction method included the major histocompatibility complex class I. Unfortunately the genome project did not assemble a complete sequence of the ovine or bovine MHC-Π locus [57-59]. Therefore, this study used only the MHC-1 alleles.

MHC-I Binding Predictions

Analysis of epitopes binding to MHC-I molecules was assessed by the software of IEDB MHC-I prediction tools. The prediction method was obtained by Artificial Neural Network (ANN), Stabilized Matrix Method (SMM) or Scoring Matrices derived from combinatorial peptide libraries [60-63]. Before the prediction step, epitopes length was set as 9mers. The conserved epitopes that bound to alleles at score equal to or less than 3 percentile rank were selected for further analysis.

Homology Modeling

Phyre2 for protein structure prediction server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index/) was used for creation the 3D structure of the VP5 and VP7 of bluetongue virus. The reference sequences [YP_052955.1 and YP_052967.1] were used as an input and Chimera 1.8 was used as a tool to visualize the selected epitopes belonging to B cell and T cell (MHC-I) [60]. Homology modeling was used for visualization of the surface accessibility of the B-lymphocytes predicted candidate epitopes as well as for visualization of all predicted T cell epitopes in the structural level [60].

Prediction of the 3D structure of the sheep allele

The sequence of sheep allele (MHC1) (b_microglobulin) was retrieved from the uniprot server at (https://www.uniprot.org/uniprot/?query=sheep+mhc1&sort=score) (uniprot number (Q6QAT4) and was used to predict the 3D structure of the sheep allele. The sequence was retrieved in FASTA format and submitted to Phre2 for homology modeling. Chimera software was used to display 3D structure of alleles

Prediction of the 3D structure of the proposed epitopes

The homology modeling of the MHC-I predicted epitopes was performed with PEP FOLD3 (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) to predict the linear structures from amino acid sequences

Molecular Docking

Molecular docking was performed according to peptide-binding groove affinity, between Sheep allele (b_microglobulin) and the proposed peptides from MHC-I. Sheep b_microglobulin allele was acted as receptors while the proposed peptides acted as ligands. Molecular docking technique of 3D structure of b_microglobulin allele and 3D modeled epitopes was performed using Patch Dock online tools; an automatic server for molecular docking (https://bioinfo3d.cs.tau.ac.il/PatchDock/) by submitting PDB of ligands and receptors after homology modeling by Phyer 2 server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) and PEP FOLD3. Firedock was used to select the best models [64, 65]. Visualization of the results was performed by the off-line UCSF-Chimera visualization tool1.8 [60].

Result

Phylogenetic Analysis

Figure (1) provided the phylogenetic relationship of the 56 and 65 retrieved strains of the VP5 and VP7 proteins of BTV viruses respectively. The phylogeny demonstrated evolutionary divergence among the retrieved strains VP5 and VP7.

Epitopes conservancy

Sequence alignment of all retrieved strains of VP5 and VP7 was performed using ClustalW that presented by Bioedit software. Sequence alignment was performed to obtain 100%-conserved epitopes from the retrieved strains. As shown in figure (2) the retrieved sequences of VP5 and VP7 demonstrated conservancy when sequences were aligned. The conserved regions were recognized by the identity of amino acid sequences among the retrieved sequences

B cell Epitopes Prediction

VP5 and VP7 reference sequences proteins were subjected to Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods from IEDB. The thresholds of Bepipred linear epitope, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity of the two proteins were shown in figure (3). In Bepipred linear epitope prediction method; the average score of VP5 protein to B-lymphocytes was -0.052 (minimum: -0.003 and maximum: 1.969). Table (2) showed that 22 epitopes were predicted by Bepipred method as a linear epitopes. In Emini surface accessibility prediction, the average score of VP5 protein was 1.000 (minimum: 0.076 and maximum: 8.010). Emini surface accessibility method predicted 15 epitopes on the surface that have potential binding to B-lymphocytes cells (Table 2). In Kolaskar and Tongaonkar antigenicity prediction method, the average score of VP5 protein was 1.019 (minimum: 0.869 and maximum: 1.222). This method predicted 9 antigenic epitopes with potential binding to B-lymphocytes cells (Table 2). Values equal to or greater than the default thresholds -0.052, 1.000 and 1.019 were considered as linear, surface accessible and antigenic epitopes, respectively. Accordingly, two conserved epitopes were successfully predicted to elicit the B cell lymphocytes since they were conserved among all retrieved strains, got higher score values in Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods. These two epitopes were 235-SEEV-235 and 85-PDPLSP-90. The three-dimensional structure (3D) level of these epitopes was shown in Figure 4.

Table 2: B-cell epitopes prediction from VP5 and VP7 proteins. The position of peptides is according to the position of amino acids in the vp5 protein of BT virus

VP5: PEPTIDES	START	END	EMINI 1.000	KOLASKAR 1.019
IPDE	294	297	1.135	0.983
GRAIE	249	253	0.666	0.963
SPKIE	275	279	1.409	1.002
SPKI	275	278	1.052	1.04
APWDS	369	373	1.029	0.98
YGESV	65	69	0.748	1.056
KDIPDE	292	297	2.278	0.955
SGRTLT	421	426	0.971	0.971
SNTAKK	21	26	2.734	0.937
RDGMQEE	209	215	2.859	0.879
EAASEEV	229	235	0.906	1.011
*SEEV	232	235	1.08	1.024
EKEIEGI	335	341	0.868	0.952
KELEDEQR	100	107	6.563	0.936
ERFAESEIGAA	36	46	0.539	0.977
GEELPDPLSPGERGMQ	81	96	1.686	0.966
*ELPDPLSPG	83	91	1.017	1.033
*ELPDPLSP	83	90	1.318	1.053
*ELPDPLS	83	89	1.083	1.051
*PDPLSP	85	90	1.485	1.053
PDPLS	85	89	1.236	1.051
LPDP	84	87	1.192	1.061
VP7: PEPTIDE	**START**	**END**	**EMINI 1.000**	**KOLASKAR 1.026**
TSLA	53	56	0.684	1.059
QGRN	176	179	2.292	0.884
AGVT	206	209	0.455	1.058
AGARGD	165	170	0.92	0.936
NPTQQN	235	240	4.873	0.926
MPGPLT	335	340	0.785	0.998
AQGNSQQT	197	204	3.118	0.96
GPISPDYTQ	78	86	2.324	1.013
*PISPDYTQ	79	86	2.836	1.03
PIFPPNDRD	297	305	3.151	0.98
*PIFPPN	297	302	1.016	1.035
EIPFTTEAANE	98	108	1.86	0.962
RVTGETSTWGPARQP	111	125	4.649	0.971

*peptides revealed higher score if they were shorten in all tools.

For VP7, Bepipred linear epitope prediction method average score was -0.012 (minimum: -0.001 and maximum: 1.719). Table (2) showed that 13 epitopes were predicted by Bepipred method as a linear epitopes. In Emini surface accessibility prediction, the average score of VP7 protein was 1.000 (minimum: 0.080 and maximum: 5.913). Emini surface accessibility method predicted 9 epitopes on the surface that have potential binding to B lymphocytes cells (Table 2). In Kolaskar and Tongaonkar antigenicity prediction method, the average score of VP7 protein was 1.026 (minimum: 0.872 and maximum: 1.228). This method predicted 4 antigenic epitopes with potential binding to B lymphocytes cells (Table 2). Values equal to or greater than the default thresholds -0.012, 1.000 and 1.026 were considered as linear, surface accessible and antigenic epitopes, respectively. Accordingly, two conserved epitopes were successfully predicted to elicit the B cell lymphocytes since they were conserved among all retrieved strains, got higher score values in Emini surface accessibility in Emini

surface accessibility and Kolaskar and Tongaonkar epitopes were 79-PISPDYTQ-86 and 297-PIFPPN-302. The three-dimensional structure (3D) level of these epitopes was shown figure 4.

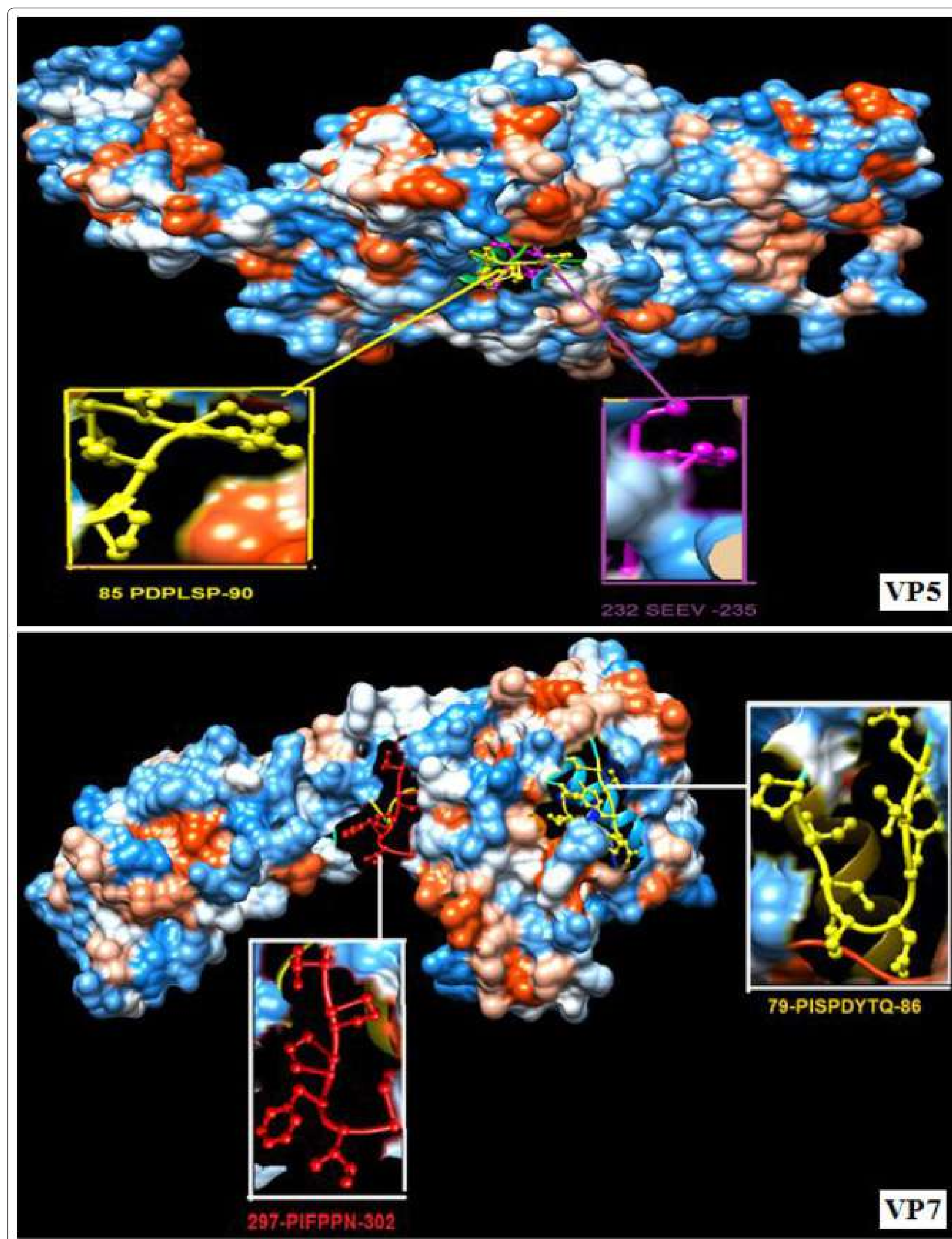

Figure 4: Position of proposed conserved B cell epitopes in structural level of VP5 and VP7 proteins of BTV. Two epitopes were predicted to interact with B cell from each protein. The epitopes showed conservancy, surface accessibility and antigenicity using IEDB software

T Lymphocytes Epitopes Prediction

VP5 protein was analyzed using IEDB MHC-1 binding prediction tool to predict T lymphocytes epitopes that have binding affinity with MHC-I alleles based on Artificial Neural Network (ANN) percentile maxi ≤ 3. As shown in Table 3, a total of 70 epitopes were found to interact with MHC-I alleles. The epitopes 257-KLKKVINAL-265, 487-QMHILRGPL-495 and 350-VMMRFKIPR-358 interacted with the highest number of alleles and demonstrated best binding favorable affinity to MHC-1 alleles. Thus were proposed as a vaccine candidate from VP5 protein. The three dimensional structural level (3D) of these epitopes within VP5 protein of BTV was shown in Figure 5.

Table 3. List of epitopes that had binding affinity with MHC-I alleles of VP5 protein. The position of peptides is according to position of amino acids in vp5 protein of BTV

Peptide	Start	End	Alleles	Percentile Rank
AERFAESEI	35	43	BoLA-HD6	1.3
			BoLA-T2b	0.84
AESEIGAAT	39	47	BoLA-D18.4	2.7
			BoLA-T2b	1.4
AIEGAYKLK	251	259	BoLA-T2a	0.68
ATIDGLVQG	46	54	BoLA-T2a	2.9
DKIDVPLFL	515	523	BoLA-D18.4	2.5
			BoLA-T2C	2.3
DKVIHPRVM	343	351	BoLA-D18.4	3
			BoLA-T2b	1.6
DLSHMRSPK	269	277	BoLA-T2a	0.69
			BoLA-T2C	1.2
DSDDVFFFH	372	380	BoLA-T2a	2.4
			BoLA-T2b	2.7
			BoLA-T2C	1.7
EAYREFLNL	427	435	BoLA-HD6	0.78
			BoLA-JSP.1	1.2
			BoLA-T2b	0.15
EEAIQEIAG	214	222	BoLA-T2b	1.3
EEVPLIGAG	233	241	BoLA-T2b	0.6
EIAGMTADV	219	227	BoLA-T2C	2.1
EPTIIATTL	279	287	BoLA-T2b	0.87
ESFFLGFDL	389	397	BoLA-HD6	3
			BoLA-JSP.1	0.22
			BoLA-T2b	0.18
FLGSMHYDI	460	468	BoLA-JSP.1	1.3
			BoLA-T2C	1.7
GAYKLKKVI	254	262	BoLA-D18.4	1.6
			BoLA-HD6	1.6
			BoLA-JSP.1	2.8
GMQEEAIQE	211	219	BoLA-D18.4	0.48
GMTADVLEA	222	230	BoLA-D18.4	2.9
GRAIEGAYK	249	257	BoLA-T2a	0.35
GSMHYDITY	462	470	BoLA-D18.4	1.5
			BoLA-JSP.1	1.3
			BoLA-T2a	0.28
HHRNESFFL	385	393	BoLA-HD6	2.2
			BoLA-T2b	0.43
			BoLA-T2C	1.6
HIKQEILPK	319	327	BoLA-T2a	0.16
HMRSPKIEP	272	280	BoLA-D18.4	0.47
HPRVMMRFK	347	355	BoLA-T2a	0.84
IEDKVIHPR	341	349	BoLA-T2b	2.6
IEGAYKLKK	252	260	BoLA-T2a	2.5

IEGIEDKVI	338	346	BoLA-T2b	2.4
IEIERDGMQ	205	213	BoLA-T2b	1.3
IEPTIIATT	278	286	BoLA-T2b	2.7
IGAATIDGL	43	51	BoLA-T2C	2.9
IHPRVMMRF	346	354	BoLA-JSP.1	1.2
IKQEILPKF	320	328	BoLA-D18.4	0.19
			BoLA-JSP.1	1.5
IKSLSRFGK	5	13	BoLA-T2a	0.43
INALSGIDL	262	270	BoLA-T2b	1.6
			BoLA-T2C	2.3
IQEIAGMTA	217	225	BoLA-D18.4	3
KEIEGIEDK	336	344	BoLA-T2a	1.5
KIIKSLSRF	3	11	BoLA-HD6	2.6
KKIYSTIGK	25	33	BoLA-T2a	0.11
KLKKVINAL	257	265	BoLA-D18.4	2.2
			BoLA-HD6	0.09
			BoLA-JSP.1	0.78
			BoLA-T2b	0.48
			BoLA-T2C	0.09
KSLSRFGKK	6	14	BoLA-T2a	0.04
KVIHPRVMM	344	352	BoLA-D18.4	0.88
			BoLA-HD6	0.2
LQMHILRGP	486	494	BoLA-HD6	2.6
LSHMRSPKI	270	278	BoLA-HD6	1.6
LTEAYREFL	425	433	BoLA-JSP.1	0.4
LVQGSVHSI	51	59	BoLA-T2C	2.3
MQEEAIQEI	212	220	BoLA-D18.4	0.07
			BoLA-HD6	0.39
PIFLGSMHY	458	466	BoLA-T2a	0.48
PKIEPTIIA	276	284	BoLA-D18.4	2.5
PTIIATTLE	280	288	BoLA-T2a	2.2
QMHILRGPL	487	495	BoLA-D18.4	0.35
			BoLA-HD6	0.05
			BoLA-JSP.1	0.03
			BoLA-T2b	0.18
			BoLA-T2C	2.1
RAIEGAYKL	250	258	BoLA-D18.4	0.28
			BoLA-HD6	0.16
			BoLA-JSP.1	2.9
RAILGALKF	500	508	BoLA-D18.4	2.4
			BoLA-HD6	2.6
RLKYNKEIT	112	120	BoLA-HD6	3
RNESFFLGF	387	395	BoLA-JSP.1	2.1
RSPKIEPTI	274	282	BoLA-HD6	1.7
			BoLA-JSP.1	0.36
RTLTEAYRE	423	431	BoLA-T2a	0.79

RVMMRFKIP	349	357	BoLA-HD6	2.5
			BoLA-T2a	2.3
SEEVPLIGA	232	240	BoLA-T2b	2.4
SGIDLSHMR	266	274	BoLA-T2a	0.74
SLSRFGKKV	7	15	BoLA-T2C	0.38
STIGKAAER	29	37	BoLA-T2a	0.12
TAKKIYSTI	23	31	BoLA-HD6	1.6
			BoLA-JSP.1	2.1
TIIATTLEH	281	289	BoLA-T2a	2.2
TLTEAYREF	424	432	BoLA-D18.4	0.27
			BoLA-T2C	0.53
VIHPRVMMR	345	353	BoLA-T2a	1.8
VMMRFKIPR	350	358	BoLA-D18.4	0.13
			BoLA-JSP.1	0.39
			BoLA-T2a	0.74
			BoLA-T2b	2.1
YKLKKVINA	256	264	BoLA-D18.4	2.9
YREFLNLSI	429	437	BoLA-JSP.1	1.1
YSTIGKAAE	28	36	BoLA-T2a	1.6

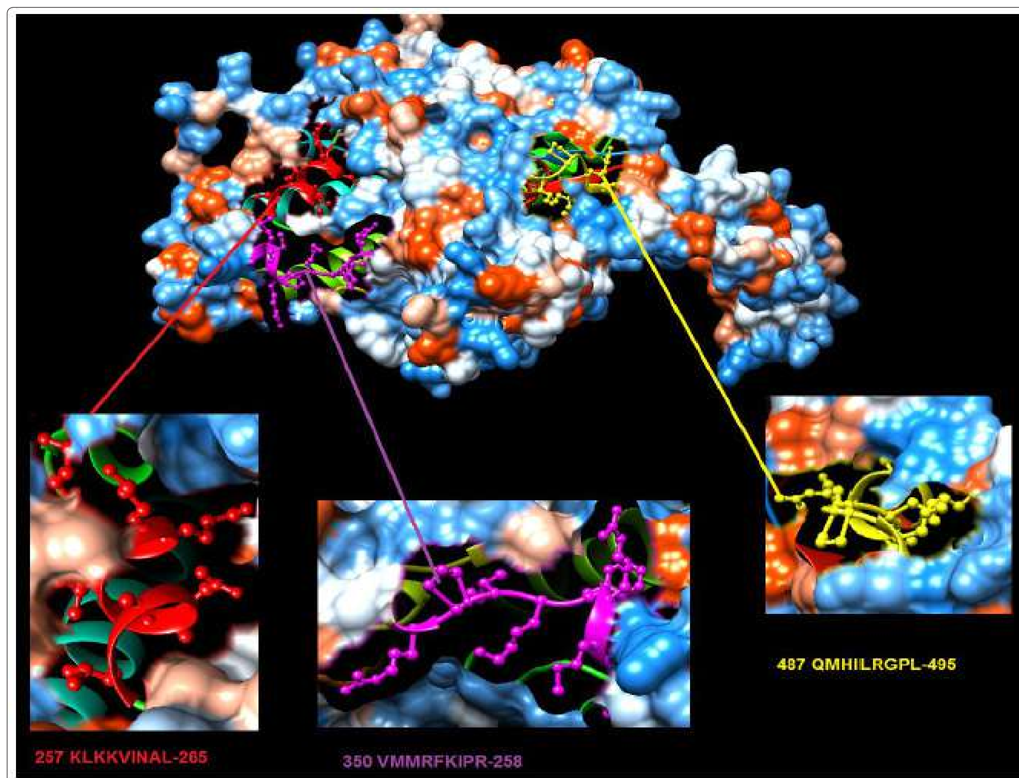

Figure 5: Position of proposed conserved T cell epitopes in structural level of VP5 protein of BTV three epitopes were predicted to interact with T cell

Vp7 protein was also analyzed using IEDB MHC-1 binding prediction tool to predict T lymphocytes epitopes that have binding affinity with MHC-I alleles based on Artificial Neural Network (ANN) percentile maxi ≤ 3. As shown in Table 4, a total of 76 epitopes were found to interact with MHC-I alleles. The epitopes 86-QHMATIGVL-94, 315-TLADVYTVL-323, 17-TLQEARIVL-25 and 10-TVMRACATL-18 interacted with the highest number of alleles provided best binding affinity to MHC-1 alleles (Table 4). The three dimensional structural level (3D) of these epitopes within VP7 of BTV was shown in Figure 6.

Table 4: List of epitopes that had binding affinity with MHC-I alleles from VP7 protein. The position of peptides is according to position of amino acids in vp7 protein of BTV

Peptide	Start	End	Allele	Pecentile Rank
AARALTVMR	5	13	BoLA-T2a	1.4
AMAQGNSQQ	195	203	BoLA-D18.4	0.47
			BoLA-HD6	2.1
ARGDVQQIF	167	175	BoLA-D18.4	1.3
ARQPYGFFL	122	130	BoLA-JSP.1	1.2
			BoLA-T2b	0.2
			BoLA-T2C	0.13
ATIGVLATP	89	97	BoLA-T2a	0.4
DMMLSAAGI	67	75	BoLA-D18.4	1.6
			BoLA-HD6	1.9
			BoLA-T2b	0.42
			BoLA-T2C	0.87
DMRAGRIIA	216	224	BoLA-D18.4	2.2
DYTQHMATI	83	91	BoLA-T2b	0.75
			BoLA-T2C	0.79
EILGIAINR	31	39	BoLA-T2a	2.7
ETSTWGPAR	115	123	BoLA-T2a	1.4
FAMAQGNSQ	194	202	BoLA-T2a	1.4
FFMCLDMML	62	70	BoLA-HD6	0.51
			BoLA-JSP.1	0.13
			BoLA-T2b	0.86
			BoLA-T2C	2.3
FQGRNDPMM	175	183	BoLA-D18.4	0.62
			BoLA-HD6	0.07
			BoLA-JSP.1	1.2
			BoLA-T2b	2.9
FRDHTWHGL	273	281	BoLA-JSP.1	3
FYISMDKTL	249	257	BoLA-HD6	1.6
			BoLA-JSP.1	0.57
GARGDVQQI	166	174	BoLA-HD6	2.3
GLTLRGVTM	42	50	BoLA-D18.4	1.2
			BoLA-HD6	1.3
			BoLA-T2b	0.37
			BoLA-T2C	2
GPARQPYGF	120	128	BoLA-T2b	2.6
GPLTRAIAR	337	345	BoLA-T2a	2.1
GRNDPMMIY	177	185	BoLA-D18.4	0.5
GVTMRPTSL	47	55	BoLA-HD6	0.77
			BoLA-T2b	0.43
HNPTQQNAM	234	242	BoLA-JSP.1	1.3
IAARALTVM	4	12	BoLA-D18.4	2.7
			BoLA-JSP.1	2
IAINRYNGL	35	43	BoLA-HD6	2.3
			BoLA-JSP.1	2.5

			BoLA-T2b	1.2
IAWDGQAAL	223	231	BoLA-D18.4	0.19
			BoLA-HD6	2.1
			BoLA-JSP.1	0.63
			BoLA-T2C	2.2
IFQGRNDPM	174	182	BoLA-JSP.1	2.1
			BoLA-T2b	0.42
INRYNGLTL	37	45	BoLA-HD6	1.9
			BoLA-JSP.1	1.9
			BoLA-T2b	1.8
IQVVFYISM	245	253	BoLA-D18.4	0.28
			BoLA-HD6	1.9
			BoLA-JSP.1	1.2
			BoLA-T2b	0.2
ISMDKTLNQ	251	259	BoLA-T2a	0.25
ISPDYTQHM	80	88	BoLA-JSP.1	1.2
KTLNQYPAL	255	263	BoLA-D18.4	0.52
			BoLA-HD6	1.2
			BoLA-JSP.1	0.33
			BoLA-T2C	0.61
LEANVMEIL	25	33	BoLA-T2b	0.23
LQEARIVLE	18	26	BoLA-HD6	2.9
LTLRGVTMR	43	51	BoLA-T2a	0.3
LTVMRACAT	9	17	BoLA-T2a	1.9
LVWRRIENF	186	194	BoLA-HD6	0.83
MIYLVWRRI	183	191	BoLA-HD6	0.73
MLPPIFPPN	294	302	BoLA-T2C	2.3
MMIYLVWRR	182	190	BoLA-D18.4	2.6
			BoLA-JSP.1	2.9
MMLSAAGIN	68	76	BoLA-D18.4	0.85
MRAGRIIAW	217	225	BoLA-D18.4	0.92
NDRDSILTL	302	310	BoLA-T2b	1.9
			BoLA-T2C	0.53
NMLPPIFPP	293	301	BoLA-D18.4	0.65
			BoLA-T2b	1.9
			BoLA-T2C	0.81
NQYPALTAE	258	266	BoLA-D18.4	0.11
			BoLA-T2C	2.8
NRTTLPNML	287	295	BoLA-JSP.1	2.2
			BoLA-T2C	1.9
PARQPYGFF	121	129	BoLA-HD6	2.9
			BoLA-T2b	1.8
PMMIYLVWR	181	189	BoLA-JSP.1	2.7
			BoLA-T2a	0.43
PPNDRDSIL	300	308	BoLA-HD6	2.7
QHMATIGVL	86	94	BoLA-D18.4	1.2
			BoLA-HD6	0.44

			BoLA-JSP.1	1.2
			BoLA-T2b	0.12
			BoLA-T2C	1.5
QYPALTAEI	259	267	BoLA-JSP.1	0.57
RAGRIIAWD	218	226	BoLA-T2a	2.4
RIIAWDGQA	221	229	BoLA-HD6	2
RIVLEANVM	22	30	BoLA-HD6	2
RNDPMMIYL	178	186	BoLA-JSP.1	0.08
RQPYGFFLE	123	131	BoLA-HD6	0.89
SMDKTLNQY	252	260	BoLA-D18.4	0.99
STWGPARQP	117	125	BoLA-T2a	2
TLADVYTVL	315	323	BoLA-D18.4	0.63
			BoLA-HD6	0.41
			BoLA-JSP.1	0.99
			BoLA-T2b	1.5
			BoLA-T2C	0.02
TLPNMLPPI	290	298	BoLA-JSP.1	0.51
			BoLA-T2C	0.88
TLQEARIVL	17	25	BoLA-D18.4	0.1
			BoLA-HD6	0.54
			BoLA-JSP.1	1.7
			BoLA-T2b	2.1
			BoLA-T2C	0.06
TMRPTSLAQ	49	57	BoLA-D18.4	0.14
TQHMATIGV	85	93	BoLA-D18.4	0.8
			BoLA-T2b	2.7
TSTWGPARQ	116	124	BoLA-T2a	1.3
TTLPNMLPP	289	297	BoLA-T2a	0.69
TVMRACATL	10	18	BoLA-D18.4	0.93
			BoLA-HD6	0.47
			BoLA-JSP.1	0.1
			BoLA-T2b	0.16
			BoLA-T2C	0.07
TWGPARQPY	118	126	BoLA-D18.4	0.51
VLATPEIPF	93	101	BoLA-D18.4	1.2
			BoLA-T2C	1.5
VLEANVMEI	24	32	BoLA-HD6	2.8
VMEILGIAI	29	37	BoLA-D18.4	1.7
			BoLA-JSP.1	1.8
VMRACATLQ	11	19	BoLA-D18.4	1.5
VSLNAGARG	161	169	BoLA-T2a	1.3
VTMRPTSLA	48	56	BoLA-T2a	1.9
VVFYISMDK	247	255	BoLA-T2a	0.06
WRRIENFAM	188	196	BoLA-D18.4	2.7
YLVWRRIEN	185	193	BoLA-D18.4	2
YSFRDHTWH	271	279	BoLA-JSP.1	2.2

			BoLA-T2a	0.13

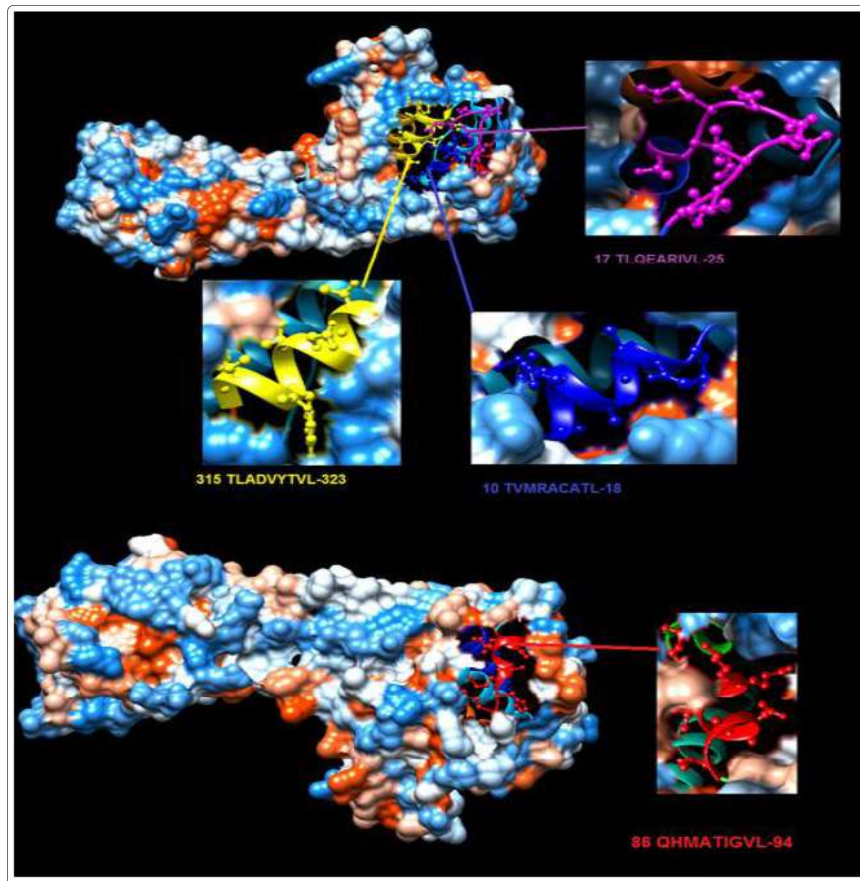

Figure 6: Position of proposed conserved T cell epitopes in structural level of VP7 protein of BTV. Four epitopes were predicted to interact with T cell

Molecular docking

Since the virus causes serious disease in sheep, the proposed epitopes were docked with MHC1 of sheep allele (b_microglobulin). As shown in table (5) the global energy binding for each proposed epitopes from that interacted with MHC-1 alleles was highly negative which indicated the strong binding features of the epitopes to the receptor.

Table 5: The global energy binding for each proposed epitopes and the attractive VDW

Ligand (VP5)	Receptor	Global energy	Attractive VDW
KLKKVINAL	b_microglobulin	-48.89	-16.79
VMMRFKIPR	b_microglobulin	-39.5	-26.38
QMHILRGPL	b_microglobulin	-51.03	-26.12
Ligand (VP7)	**Receptor**	**Global energy**	**Attractive VDW**
TLQEARIVL	b_microglobulin	-44.78	-21.22
TLADVYTVL	b_microglobulin	-45.45	-23.38
TVMRACATL	b_microglobulin	-48.69	-22.02
QHMATIGVL	b_microglobulin	-46.25	-20.85

The top ranked proposed epitopes were selected for molecular docking to predict and symbolize the image of real MHC1 epitopes interaction with sheep allele (b macroglobulin). For VP5 and VP7; the peptide-binding groove affinity was used to evaluate the ability of proposed epitopes to bind the sheep alleles/receptors. Results indicated that the docked epitopes achieved strong binding affinity to sheep alleles based on global energy and attractive VDW in kcal/mol unit (table 5). The lowest binding energy (kcal/mol) was selected to predict MHC1 probable epitopes. Docked ligand epitopes of VP5 (257-KLKKVINAL-265, 350-VMMRFKIPR-358, 487-QMHILRGPL-495) with b_microgobulin allele (receptor) showed higher binding affinity which expressed by the lower

global energy (-48.89, -39.50 and -51.03 respectively). For VP7, the ligand epitopes (17-TLQEARIVL-25, 315-TLADVYTVL-323, 10-TVMRACATL-18 and 86-QHMATIGVL-94) when docked with receptor b_microglobulin showed higher binding affinity which expressed by the lower global energy (-44.78, -45.45, -48.69 and -46.25, respectively).

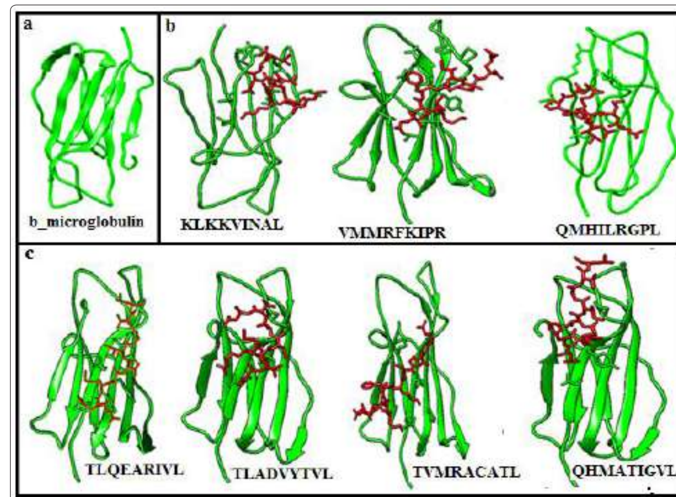

Figure 7: Visualization of PatchDock molecular docking of (a): b_microglobulin alleles receptors (b): VP5 proposed epitopes to MHC-I and (c): VP7 proposed epitopes to MHC-I using UCSF-Chimera visualization tools. The receptors (b_microglobulin alleles) were represented by green colour while CTL epitopes were represented by red colour

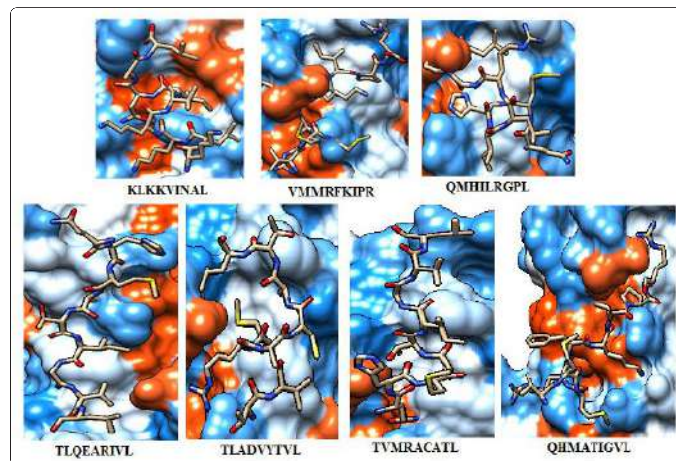

Figure 8: Visualization of PatchDock molecular docking of MHCI proposed epitopes and b_microglobulin alleles receptors using UCSF-Chimera visualization tool for both VP5 (the upper epitopes) and VP7 (the lower epitopes). The receptors (b_microglobulin alleles) represented by rounded ribbon structure hotpink colour while CTL epitopes were represented by sticks structure

Figure 7 & 8 provided the 3D structure of the sheep allele and the proposed epitopes binding sites. The visualization of the binding interactions between the sheep allele (receptor) and the proposed epitopes from VP5 and VP7 in the structural level was performed using UCSF-Chimera visualization tool 1.8.

Discussion

Bluetongue virus (BTV) causes the hemorrhagic disease bluetongue (BT) in ruminants. Currently the control of the disease outbreaks is through vaccination with conventionally modified-live and inactivated vaccines [66, 67]. However, these vaccines demonstrated multiple drawbacks such as the high costs of production, the need for multiple priming doses of vaccine as well as booster immunizations. Inactivated vaccines also have inherent potential limitations due to stability and product ‘‘shelf life,’’ and these limitations may limit their utility in outbreak situations [68]. The ideal BT vaccine is efficacious, safe, affordable, and protective against multiple serotypes and enables the differentiation of infected from vaccinated animals. Therefore, there is a need for improved BT vaccines. In this study, we used various bioinformatics tools to predict multiple epitopes from VP5 and VP7 proteins of the BTV against B and T cells. Both humoral and cellular immunity contribute to protection against BTV infection and an effective vaccine should, therefore, aim to induce both [69, 70].

The purpose from B cell epitope prediction was to find the potential antigen that would interact efficiently with B cells and eliciting immune response. For the epitope to be a B cell epitope, it would be a linear epitope, has surface accessibility and antigenic as well [54-56]. These criteria were performed by IEDB prediction tools. Two conserved epitopes (235-SEEV-235 and 85-PDPLSP-90) from the VP5 protein and another two conserved epitopes (79-PISPDYTQ-86

and 297-PIFPPN-302) from VP7 were successfully proposed eliciting the B cell lymphocytes since they were conserved linear epitopes among all retrieved strains, got higher score values in Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods.

T cells have crucial role in stimulation of immune response as well as antigen mediated clonal expression of B cell. The IEDB server did not assemble a complete sequence of the bovine or ovine MHC-Π locus. Thus the analysis was only completed with BoLA MHC-I alleles. Cytotoxic CD8+T lymphocytes (CTL) restrict the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines [71, 72]. Thus, T-Cell epitope-based vaccination is a unique process of eliciting strong immune response against infectious agents such as viruses [73]. The cytotoxic T cells are responsible for the immune elimination of intracellular pathogens such as viruses because these cells recognize the presented endogenous antigenic peptides by the MHC class I molecules. In this study, MHC-1 binding prediction methods using IEDB database predicted different CTLs epitopes that strongly interacted with various BoLA alleles. For instance a total of 70 and 76 epitopes from VP5 and VP7 proteins were found to interact with MHC-I alleles, respectively. The epitopes 257-KLKKVINAL-265, 487-QMHILRGPL-495 and 350-VMMRFKIPR-358 from VP5 protein and 86-QHMATIGVL-94, 315-TLADVYTVL-323, 17-TLQEARIVL-25 and 10-TVMRACATL-18 from VP7 protein were found interacted with the highest number of alleles and demonstrated best binding affinity to MHC-1 alleles. Accordingly, these epitopes were proposed as a candidate vaccine against BTV disease.

To study the effective binding between the MHC-1 proposed epitopes from the VP5 and VP7 proteins with ovine HLA molecules, the proposed epitopes were docked against sheep allele (b_macroglobulin). The docked ligand epitopes of VP5 (257-KLKKVINAL-265, 350-VMMRFKIPR-358, 487-QMHILRGPL-495) with b_microgobulin allele (receptor) showed higher binding affinity which expressed by the lower global energy (-48.89, -39.50 and -51.03) respectively. For VP7 epitopes (17-TLQEARIVL-25, 315-TLADVYTVL-323, 10-TVMRACATL-18 and 86-QHMATIGVL-94) when docked with b_microgobulin allele (receptor) expressed lower global energy (-48.89, -39.50 and -51.03), respectively. These docking results favored the good binding affinity between sheep allele and the proposed epitopes of MHC-1.

Conclusion

This study focused mainly on the production of a peptide vaccine from VP5 and VP7 proteins of BTV disease using immnunoiformatics approach. Epitopes that showed conservancy and high binding affinities to many MHC-1 alleles were proposed as the best candidates for vaccine production. Although bioinformatics studies have been established to facilitate the peptide vaccine design, not all the in silico predicted epitopes are optimally immunogenic in vivo. Thus, it remains necessary to test the expected peptides in vivo to ensure their efficacy to elicit both B and T cells.

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Competing Interest

The authors declare that they have no competing interests.

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References

1. MacLachlan NJ, Drew CP, Darpel KE, Worwa G (2009) The pathology and pathogenesis of bluetongue. J Comp Pathol 141: 1-16.
2. Coetzer JAW, Tustin RC (2004) Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa. Cape Town 2004: 1201-1220.
3. Mertens PP, Brown F, Sangar DV (1984) Assignment of the genome segments of bluetongue virus type 1 to the proteins, which they encode. Virology 135: 207-217.
4. Mertens PPC (1986) The genome segments of bluetongue virus: the proteins theyencode and the relationship between different isolates by cross-hybridization. Rev Sci Tech Off Int Epiz 5: 333-349.
5. Roy P (1989) Bluetongue virus genetics and genome structure. Virus Res 13: 179-206
6. Spreull J (1905) Malarial catarrhal fever (bluetongue) of sheep in South Africa. J Comp Pathol Ther 18: 321-337.
7. Savini G, Lorusso A, Paladini C, Migliaccio P, Di Gennaro A, et al. (2014) Bluetongue serotype 2 and 9 modified live vaccine viruses as causative agents of abortion in livestock: a retrospective analysis in Italy. Transbound Emerg Dis 61: 69-74.
8. Rasmussen LD, Savini G, Lorusso A, Bellacicco A, Palmarini M, et al. (2013) Transplacental transmission of field and rescued strains of BTV-2 and BTV-8 in experimentally infected sheep. Vet Res 44: 75.
9. van der Sluijs MT, de Smit AJ, Moormann RJ (2016) Vector independent transmissionof the vector-borne bluetongue virus. Crit. Rev. Microbiol 42: 57-64.
10. Maan S, Maan NS, Nomikou K, Batten C, Antony F, et al. (2011) Novel bluetongue virus serotype from Kuwait. Emerg Infect Dis 17: 886-889.
11. Batten C, Henstock MR, Steedman HM, Waddington S, Edwards L, et al. (2013) Bluetongue virus serotype 26: infection kinetics, pathogenesis and possible contact transmission in goats. Vet Microbiol 162: 62-67.
12. Batten C, Darpel K, Henstock M, Fay P, Veronesi E, et al. (2014) Evidence for transmission of bluetongue virus serotype 26 through direct contact. PLoS One 9: e96049.
13. MacLachlan NJ, Drew CP, Darpel KE, Worwa G (2009) The pathology and pathogenesis of bluetongue. J Comp Pathol 141: 1-16.
14. Schwartz-Cornil I, Mertens PP, Contreras V, Hemati B, Pascale F, et al. (2008) Bluetongue virus: virology, pathogenesis and immunity. Vet Res 39: 46.
15. Coetzer JAW, Tustin RC (2004) Infectious diseases of livestock, 2nd ed., Oxford University Press southern Africa. Cape Town 2004: 1201-1220.
16. De Clercq K, De Leeuw I, Verheyden B, Vandemeulebroucke E, Vanbinst T, et al. (2008) Transplacental infection and apparently immunotolerance induced by a wild-type bluetongue virus serotype8 natural infection, Transbound. Emerg Dis 55: 352-359.
17. Wouda W, Roumen MP, Peperkamp NH, Vos JH, van Garderen E, et al. (2008) Hydranencephaly in calves following the

bluetongue serotype 8epidemic in the Netherlands. Vet Rec 162: 422-423.
18. Wouda W, Peperkamp NH, Roumen MP, Muskens J, van Rijn A, et al. (2009) Epizootic congenital hydranencephaly and abortion in cattledue to bluetongue virus serotype 8 in the Netherlands, Tijdschr. Diergeneeskd 134: 422-427.
19. Bonneau KR, MacLachlan NJ (2004) Genetic diversification of field strains of bluetongue virus. Vet Ital 40: 446-447.
20. Maan S, Maan NS, van Rijn PA, van Gennip RGP, Sanders A, et al. (2010) Full genome characterisation of bluetongue virus serotype 6 from the Netherlands 2008 and comparison to other field and vaccine strains. PLoS One 5: e10323.
21. International Committee on Taxonomy of Viruses (2011) Virus taxonomy: 9th report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford, United Kingdom
22. Roy P, Urakawa T, Van Dijk AA, Erasmus BJ (1990) Recombinant virus vaccine for bluetongue disease in sheep. J Virol 64: 1998-2003.
23. Huismans H, Erasmus BJ (1981) Identification of the serotype-specific and group-specific antigens of bluetongue virus. Onderstepoort J Vet Res 48: 51-58.
24. Inumaru S, Roy P (1987) Production and characterization of the neutralization antigen VP2 of bluetongue virus serotype 10 using a baculovirus expression vector. Virology 157: 472-479.s
25. Gumm ID, Newman JF. 1982. The preparation of purified bluetonguevirus group antigen for use as a diagnostic reagent. Arch Virol 72: 83-93.
26. Perrin A, Albina E, Bréard E, Sailleau C, Promé S, et al. (2007) Recombinantcapripoxviruses expressing proteins of bluetongue virus: evaluation of immune responses and protection in small ruminants. Vaccine 25: 6774-6783.
27. Jabbar TK, Calvo-Pinilla E, Mateos F, Gubbins S, Bin-Tarif A, et al. (2013) Protection of IFNAR mice againstbluetongue virus serotype 8, by heterologous (DNA/rMVA) and homologous (rMVA/rMVA) vaccination, expressing outer-capsid protein VP2.PLoS One 8: e60574.
28. Janardhana V, Andrew ME, Lobato ZI, Coupar BE (1999) The ovinecytotoxic T lymphocyte responses to bluetongue virus. Res Vet Sci 67: 213-221.
29. Roy P, Bishop DH, LeBlois H, Erasmus BJ (1994) Long-lasting protection of sheep against bluetongue challenge after vaccination with virus-like particles: evidence forhomologousandpartial heterologous protection. Vaccine 12: 805-811.
30. Jones LD, Chuma T, Hails R, Williams T, Roy P (1996) The nonstructuralproteins of bluetongue virus are a dominant source of cytotoxic T cell peptide determinants. J Gen Virol 77: 997-1003.
31. Jones LD, Williams T, Bishop D, Roy P (19970 Baculovirus-expressednonstructural protein NS2 of bluetongue virus induces a cytotoxic T-cellresponse in mice which affords partial protection. Clin Diagn Lab Immunol 4: 297-301.
32. Anderson J, Mertens PP, Herniman KA (1993) A competitive ELISA forthe detection of anti-tubule antibodies using a monoclonal antibodyagainst bluetongue virus non-structural protein NS1. J Virol Methods 43: 167-175.
33. Andrew M, Whiteley P, Janardhana V, Lobato Z, Gould A, et al. (1995) Antigen specificity of the ovine cytotoxic T lymphocyte response tobluetongue virus. Vet Immunol Immunopathol 47: 311-322.
34. Barros SC, Cruz B, Luís TM, Ramos F, Fagulha T, et al. (2009) A DIVA system based on the detection of antibodiesto non-structural protein 3 (NS3) of bluetongue virus. Vet Microbiol 137: 252-259.
35. Theiler A (1908) The inoculation of sheep against bluetongue and the results inpractice. Vet J 64: 600-607.
36. Savini G, MacLachlan NJ, Sanchez-Vizcaino JM, Zientara S (2008) Vaccinesagainst bluetongue in Europe. Comp Immunol Microbiol Infect Dis 31: 101-120.
37. Veronesi E, Darpel KE, Hamblin C, Carpenter S, Takamatsu HH, Anthony SJ, et al. (2010) Viraemia and clinical disease in Dorset Poll sheep following vaccination with live attenuated bluetongue virus vaccinessero types 16 and 4. Vaccine 28: 1397-1403.
38. Gethmann J, Huttner K, Heyne H, Probst C, Ziller M, et al. (2009) Comparative safety study of three inactiv-ated BTV-8 vaccines in sheep and cattle under field conditions. Vaccine 27: 4118-4126.
39. Calvo-Pinilla E, Rodriguez-Calvo T, Anguita J, Sevilla N, Ortego J (2009a) Establishment of a bluetongue virus infection model in mice that are deficient in the alpha/beta interferon receptor. PLOS ONE 4: e5171.
40. Calvo-Pinilla E, Rodriguez-Calvo T, Anguita J, Sevilla N, Ortego J (2009b) Heterologousprime boost vaccination with DNA and recombinant modified vaccinia virus Ankara protects IFNAR(−/−) mice against lethal bluetongue infection. Vaccine 28: 437-445.
41. Calvo-Pinilla E, Navasa N, Anguita J, Ortego J (2012) Multiserotypeprotectionelicited by a combinatorial prime-boost vaccination strategy against bluetonguevirus. PLOS ONE 7: e34735.
42. Jabbar TK, Calvo-Pinilla E, Mateos F, Gubbins S, Bin-Tarif A, et al. (2013) Protection of IFNAR (−/−) mice against bluetongue virusserotype 8, by heterologous (DNA/rMVA) and homologous (rMVA/rMVA) vacci-nation, expressing outer-capsid protein VP2. PLOS ONE 8: e60574.
43. De Groot AS, Sbai H, Aubin CS, Julie McMurry, William Martin (2002) Immuno-informatics: mining genomes for vaccine components. Immunol Cell Biol 80: 255-269.
44. Moutschen M, Léonard P, Sokal EM, Françoise Smets, Michèle Haumont, et al. (2007) Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. Vaccine 25: 4697-4705.
45. Goepfert PA, Tomaras GD, Horton H, David Montefiori, Guido Ferrari, et al. (2007) Durable HIV-1 antibody and T-cell responses elicited by an adjuvanted multi-protein recombinant vaccine in uninfected human volunteers. Vaccine 25: 510-518.
46. Mancini-Bourgine M, Fontaine H, Bréchot C, Stanislas Pol, Marie-Louise Michel (2006) Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. Vaccine 24: 4482-4489.
47. Nemunaitis J, Meyers T, Senzer N, Casey Cunningham, Howard West, et al. (2006) Phase I Trial of sequential administration of recombinant DNA and adenovirus expressing L523S protein in early stage non-small-cell lung cancer. MolTher 13: 1185-1191.
48. Chianese-Bullock KA, Irvin WP, Petroni GR, Cheryl Murphy, Mark Smolkin, et al. (2008) A multipeptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer. J Immunother 31: 420-430.
49. Slingluff CL, Petroni GR, Chianese-Bullock KA, Mark E Smolkin, Sarah Hibbitts, et al. (2007) Immunologic and clinical outcomes of a randomized phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. Clin Cancer

Res 13: 6386-6395.
50. National Center for Biotechnology Information, NCBI. https://www.ncbi.nlm.nih.gov/protein/?term=bluetongue+vp5,vp7
51. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6:molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725-2729.
52. Hall T (1999) A BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98.
53. Immune Epitope Database (IEDB) analysis. http://www.iedb.org/
54. Jespersen MC, Peters B, Nielsen M, Marcatili P (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 45: W24-W29.
55. Emini EA, JV Hughes, DS Perlow, J Boger (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol Sep 55: 836-839.
56. Kolaskar AS, Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 276: 172-174.
57. Nene V, Nicholas Svitek, Philip Toye, William T Golde, John Barlow, et al. (2012) Designing bovine T cell vaccines via reverse immunology. Ticks and tick-borne diseases 3: 188-192.
58. Sequencing BG, A Consortium, Elsik CG, Tellam RL, Worley KC, et al. (2009) The genome sequence of taurine cattle: A window to ruminant biology and evolution. Science 324: 522-528.
59. Zimin AV, Arthur L Delcher, Liliana Florea, David R Kelley, Michael C Schatz, et al. (2009) A whole-genome assembly of the domestic cow, Bos taurus. Genome biology 10: R42.
60. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, et al. (2004) UCSF Chimera a visualization system for exploratory research and analysis. J Comput Chem 25: 1605-1612.
61. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10: 845-858.
62. Schneidman-Duhovny D, Y Inbar, R Nussinov, HJ Wolfson (2005) “Patch Dock and symmetric docking.” Nucleic Acids Research 33: W363-W367.
63. Andrusier N, R Nussinov, HJ Wolfson (2007) “FireDock: Fast interaction refinement in molecular docking.” Proteins: Structure, Function, and Genetics 69: 139-159.
64. Schneidman-Duhovny D, Y Inbar, R Nussinov, HJ Wolfson (2005) Patch Dock and Symm Dock: servers for rigid and symmetric docking. Nucleic Acids Research 33: W363-W367.
65. Shen Y, J Maupetit, P Derreumaux, P Tuffery (2014) Improved PEP-FOLD approach for peptide and miniprotein structure prediction.” Journal of Chemical Theor yan dComputation 10: 4745-4758.
66. Stott JL, Barber TL, Osburn BI (1985) Immunologic response of sheep to inactivated and virulent bluetongue virus. Am J Vet Res 46: 1043-1049.
67. Di Emidio B, Nicolussi P, Patta C, Ronchi GF, F Monaco, et al. (2004) Efficacy and safety studies on an inactivated vaccine against bluetongue virus serotype 2. Vet Ital 40: 640-644.
68. McVey SD, MacLachlan NJ (2015) Vaccines for Prevention of Bluetongue and Epizootic Hemorrhagic Disease in Livestock: A North American Perspective. Vector-borne and zoonotic diseases 15: 385-396.
69. eggo MH, Wardley RC, Brownlie J (1985) Importance of ovine cytotoxic T cells in protection against bluetongue virus infection. Prog Clin Biol Res 178: 477-487.
70. Jeggo MH, Wardley RC, Taylor WP (1984) Role of neutralising antibody in passive immunity to bluetongue infection. Res Vet Sci 36: 81-86.
71. Garcia KC, Teyton L, Wilson IA (1999) Structural basis of T cell recognition. Annu Rev Immunol 17: 369-397.
72. Sharmeen R, Hossain MN, Rahman MM, Foysal MJ, Miah MF (2012) In-vitro antibacterial activity of herbal aqueous extract against multi-drug resistant Klebsiella sp. isolated from human clinical samples. Int Curr Pharm J 1: 133-137.
73. Shrestha B (2004) Role of CD8+ T cells in control of West Nile virus infection. J Virol 12: 8312-8321.

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