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B and T-Cell Epitope Prediction of the OMP25 Antigen for Developing *Brucella melitensis* Vaccines for Sheep

Research Article

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ABSTRACT

Brucellosis, produced by *Brucella* species, is a disease that causes severe economic losses for livestock farms worldwide Due to serious economic and medical consequences of this disease, many efforts have been made to prevent the infection through the use of recombinant vaccines based on *Brucella* outer membrane protein (OMP) antigens. In the present study, a wide range of on-line prediction software was used to predict B and T-cells epitopes, secondary and tertiary structure and antigenicity OMP25 antigens. The bioinformatics approach used in the present study was validated by comparing its results with four available experimental epitope predictions. Bioinformatics analysis identified B-cell epitopes locations at amino acid (AA) residues 26-44, 59-79, 88-112, 146-166 and 175-202l and T-cell epitopes at AA residues 1-10, 14-22, 122-132, 154-162 and 206-213. All final B and T-cell predicted epitopes, except 1-10 and 14-22 residuals, showed antigenicity ability. Finally, a common B and T-cell epitope was identified at 154-162 of the OMP25 antigen. Bioinformatics analysis showed that this region has proper epitope characterization and so may be useful for producing recombinant vaccine.

KEY WORDS *Brucella*, epitope prediction, OMP25.

INTRODUCTION

Brucellosis is a widespread zoonotic disease that still of veterinarian, public health and economic concern in many developing countries (Karthik *et al*. 2013). This disease is caused by bacteria of the genus *Brucella*, Gram-negative, facultative, intracellular pathogens that can infect primarily the domestic animals (Cutler and Whatmore, 2005). The main worldwide pathogenic species are *Brucella abortus* and *Brucella melitensis*, which are involved in bovine and ovine brucellosis, respectively (Corbel and Brinley-Morgan, 1984). These diseases are characterized by abortion and reduced fertility in animals and also by chronic infections with symptoms such as undulant fever, arthritis and osteomyelitis in humans (Pappas *et al*. 2006). Brucella outer membrane proteins (OMPs) play a critical role in stimulating host immune system. The major *Brucella* OMPs have been classified in two groups 1:OMP2a and OMP2b (36-38 kDa) and 2:OMP25 and OMP31 (25 kDa and 31-34 kDa) (Cloeckaert *et al*. 2002). OMP25 has been reported to be immunodominant and conserved in pathogenic *Brucella* species (Cloeckaert *et al*. 1996; Cloeckaert *et al*. 2002). There are several reports have shown that OMP25 recombinant protein and DNA vaccine is protective against the virulent challenge of *Brucella* species in mice (Commander *et al*. 2007; Goel and Bhatnagar, 2012). This makes the OMP25 as a valuable target for epitope-based vaccine design. When the host encounters a pathogenic virus or bacterium, it may produce specific antibodies which bind to sites on the surface of antigens that known as antigenic determinants or epitopes (Berzofsky, 1985). Epitopes classified as B and T-cell epitopes (Almeida *et al.* 2012; Zhang *et al.*

2012). B-cell epitopes can be divided in two categories (i) continuous and (ii) discontinuous. The continuous (linear epitopes) are made up of consecutive amino acids whereas the discontinuous (conformational epitopes) constitute the spatially folded amino acids, which lie far away in the primary sequence. The majority of peptides examined as vaccine candidates correspond to continuous epitopes (Chen *et al.* 2011).

T-cell epitopes, as presented in the major histocompatibility complex (MHC) molecule are antigenic peptide strings recognized by T-cells receptors. The MHCI molecule binds to a peptide with approximately 9 AA within a closed groove.

In contrast, because the antigen-binding groove is open at both ends, MHCII molecules can present much longer peptides, generally varying from 12 to 25 amino acids (Chen *et al.* 2011). Computational tools can be used for the prediction of B and T-cell epitopes for their use in antibody production, immunodiagnostics and epitope-based vaccine design (Dudek *et al.* 2010; Bryson *et al.* 2010; Steere *et al.* 2011).

Bioinformatics approaches are relatively rapid and inexpensive and can be used, at least in part to replace by experimental methods (Ponomarenko and van Regenmortel, 2009).

Several epitopes prediction software programs are currently available. The first generation of these prediction tools performed base on motif-based algorithms (Chen *et al.* 2011), antigen primary amino acid sequence (Hopp and Woods, 1981), 3D structure and other protein characteristics such as hydrophilicity, accessibility and flexibility (Kyte and Doolittle, 1982; Karplus and Schulz, 1985; Hopp and Woods, 1981).

Recently more sophisticated methods using various machine learning based algorithms have been developed based on support vector machines (SVM) (Donnes and Elofsson, 2002), hidden markov models (HMM) (Noguchi *et al.* 2002) and artificial neural networks (ANN) (Buus *et al.* 2003).

Devasundaram *et al.* (2014) detected four T-cell epitopes for the development of subunit vaccines against tuberculosis using *in silico* analysis (Propred I and Propred software). In this regards, Ghasemi *et al.* (2014) analyzed chimeric TF, Omp31 and BP26 fragments of *Brucella melitensis* for development of a multi subunit vaccine candidate using *in silico* approaches. In addition, Pavlović *et al.* (2014) predicted B and T-cell epitopes for Epstein Barr Virus using bioinformatics software. In the present study, with the aim of epitopic-based vaccine design, at first the predictive ability of the bioinformatics tools were tested using four different sequences and then their results were compared with the experimental results from IEDB for validation. After testing and validation, OMP25 B and T-cell epitopes were predicted.

MATERIALS AND METHODS

Amino acid sequence of the OMP25 protein

The nucleotide sequence of OMP25 (AEF59022.1) was obtained from GenBank (http://www.ncbi.nih.gov/genbank/).

The OMP25 protein is composed of 213 amino acid residues (Figure 1). Structures and B and T-cell epitopes of the *Brucella* secondary and tertiary structures as well as B and T-cell epitopes of the *Brucella* OMP25 protein were predicted using the bioinformatics software listed in Table 1. A summary of bioinformatics approach was illustrated in Figure 2.

Prediction of the secondary structure of the OMP25 protein

The secondary structure of the OMP25 protein was predicted using the improved self-optimized prediction method (SOPMA) software (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) (Geourjon and Deléage, 1995).

Four conformational states (helices, sheets, turns and coils) of OMP25 were analyzed.

Prediction of the tertiary structure of the OMP25 protein

Predictive analysis of the OMP25 protein tertiary structure was conducted using 3DLigandSite (http://www.sbg.bio.ic.ac.uk) (Wass *et al.* 2010). This web server automates the manual processes used for the prediction of ligand-binding sites in the eighth round of the critical assessment of protein structure prediction (CASP8) (Wass and Sternberg, 2009).

Confirming the bioinformatics analysis approach

In order to confirm our predicted outputs, the results of four different experimental epitope prediction studies were evaluated by bioinformatics tools were used in present study. Antigen name and their reference were listed in Table 2.

Characterization of epitopes

Final B and T-cell predicted epitopes were evaluated using the VaxiJen 2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) for the alignment-independent prediction of protective antigens. In addition, enzymatic degradation sites, Mass (Da) and pI were determined using the Protein Digest server (http://db.systemsbiology.net:8080/proteomicsToolkit/proteinDigest.html).

MRTLKSLVIVSAALLPFSATAFAADAIQEQPPVPAPVEVAPQYSWAGGYTGLYLGYGW
NKAKTSTVGSIKPDDWKAGAFAGWNFQQDQIVYGVEGDAGYSWAKKSKDGLEVKQG
FEGSLRARVGYDLNPVMPYLTAGIAGSQIKLNNGLDDESKFRVGWTAGAGLEAKLTDN
ILGRVEYRYTQYGNKNYDLAGTTVRNKLDTQDFRVGIGYKF

Figure 1 Amino acid sequence of the OMP25 protein

Table 1 Bioinformatics software that used in present study

Name	Description	Link
T cell epitopes prediction		
IEDB	SVM and ANN-based method for prediction	http://tools.immuneepitope.org/mhci
SYFPEITH	A database of MHC ligands and peptide motifs; predictive server for MHC binding peptide	http://www.syfpeithi.de
NetMHC	ANN-based method for prediction of HLA	http://www.cbs.dtu.dk/services/NetMHC
NetCTL	Prediction of T-cell epitope	http://www.cbs.dtu.dk/services/NetCTL
PropredI	predict MHC class I binding peptides	http://www.imtech.res.in/raghava/propred1
Propred	predict MHC class II binding peptides	http://www.imtech.res.in/raghava/propred
B cell epitopes prediction		
Bcepred	Physio-chemical properties of amino acids based predictive server for linear B cell epitope	http://www.imtech.res.in/raghava/bcepred
ABCpred	ANN based predictive server	http://www.imtech.res.in/raghava/abcpred
BepiPred	Predictor of linear B cell epitopes using a combination of a hidden Markov model and a propensity scale method	http://www.cbs.dtu.dk/services/BepiPred
BCPred	Prediction of linear B-cell epitopes using amino acid pair anti-genicity scale and string kernels	http://ailab.cs.iastate.edu/bcpreds
SVMTrip	Predictor of linear B cell epitopes using Support Vector Machine (SVM)	http://sysbio.unl.edu/SVMTriP
LEPS	Prediction of linear B-cell epitopes using Support Vector Machine classification and amino acid propensity	http://leps.cs.ntou.edu.tw
IEDB	Physio-chemical properties of amino acids based predictive server for linear B cell epitope	http://tools.immuneepitope.org/tools/bcell/iedb_input

RESULTS AND DISCUSSION

Confirming the bioinformatics analysis approach

In order to train all of the software used in the present study four antigens, whose epitopes were determined experimentally (http://www.iedb.org), were selected and their epitopes were predicted using bioinformatics tools. The predicted epitopes compared with the results of experimental researches. Results revealed that our *in sillico* predicted epitopes were similar to the founding of experimental results for all of the selected antigens (Table 2).

Prediction of the B-cell epitopes for the OMP25 antigen

The B-cell epitope predictions were performed using different online software listed in Table 3. For each software highest score epitopes were selected as appropriate epitopes. Moreover, five epitopes were chosen as"final B-cell epitopes" by identifying those epitopes which had most conserved sequences in all proposed epitopes (Table 4).

Prediction of the T-cell epitopes for the OMP25 antigen

The results of MHCI (A-0101, A0201 and B-2705) and MHCII (DRB1-0101 and DRB1-0401) class of T-cell epitope prediction are shown in Table 5.

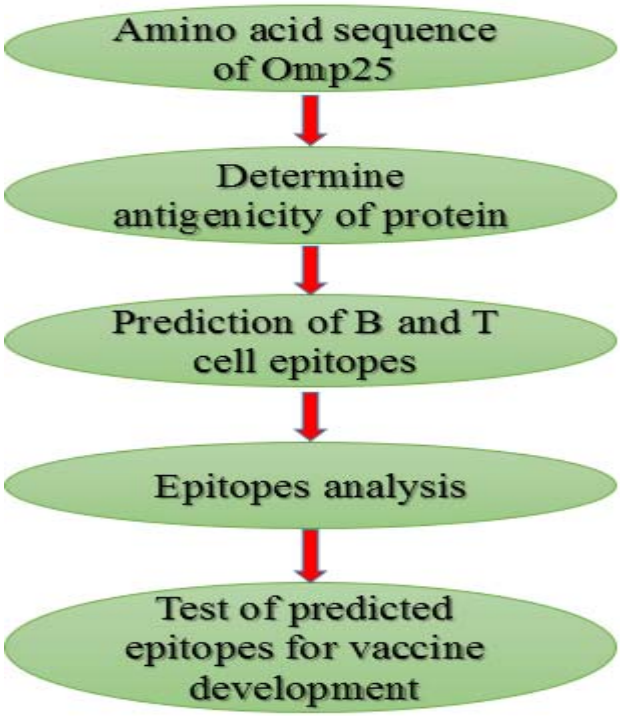

Figure 2 A summary of research steps

Table 2 Training bioinformatics software that used in present study

Antigen	Predicted epitopes	Experimental epitopes	Reference
GroEL[1] of *Yarsinia*	28-42,78-92,178-185,275-290,**315-336**,430-440,526-545	316-326	Yamaguchi *et al*. (1996)
Dnak[2] of *Brucella*	40-67,78-92,210-227,357-370, 523-537, **609-640**	617-637	Vizcaino *et al*. (1997)
Omp31[3] of *Brucella*	25-28,**46-73**,122-127,175-182	48-74	Wang *et al*. (2014) and Cassaratro *et al*. (2005)
SOD[4] of *Brucella*	44-50**,70-86,134-153,147-165**	75-86,136-150,149-162	Tabatabai *et al*. (1994)

[1] Heat shock protein 60; [2] Heat shock protein 70; [3] Outer membrane 31 and [4] Sodium oxide dismutase.
Similar epitopes between predicted epitopes using bioinformatics tools and experimental studies were specified by bold and underline.

Table 3 B-cell predicted epitopes with highest score using different servers

Server		NO.	Sequence	Score
		1	$_{103}$KKSKDGLEVK$_{112}$	1.83
		2	$_{149}$LDDES$_{154}$	1.74
Bcepred		3	$_{60}$KAKTSTVGSIKPDDWK$_{75}$	1.46
		4	$_{88}$QIVYGVEGDAG$_{98}$	1.32
		5	$_{200}$LDTQDFRV$_{207}$	1.25
		1	$_{26}$AIQEQPPVPAPVEVAPQYSWAG$_{47}$	1.46
BepiPred		2	$_{59}$NKAKTSTVGSIKPDDWK$_{75}$	0.98
		3	$_{93}$VEGDAGYSWAKKS$_{105}$	0.85
		4	$_{182}$TQYGNKNYDLAGT$_{194}$	0.74
		1	$_{33}$VPAPVEVAPQYSWAGGYTGL$_{52}$	1.00
		2	$_{59}$NKAKTSTVGSIKPDDWKAGA$_{78}$	1.00
	AAP	3	$_{89}$IVYGVEGDAGYSWAKKSKDG$_{108}$	1.00
		4	$_{111}$VKQGFEGSLRARVGYDLNPV$_{130}$	1.00
		5	$_{176}$VEYRYTQYGNKNYDLAGTT$_{195}$	1.00
		6	$_{146}$NNGLDDESKFRVGWTAGAGLE$_{166}$	0.91
		1	$_{26}$AIQEQPPVPAPVEVAPQYSW$_{45}$	1.00
BCPREDS	**BCPred**	2	$_{57}$GWNKAKTSTVGSIKPDDWKA$_{76}$	1.00
		3	$_{89}$IVYGVEGDAGYSWAKKSKDG$_{108}$	0.89
		1	$_{31}$PPVPAPVEVAPQYS$_{44}$	1.00
		2	$_{95}$GDAGYSWAKKSKDGL$_{109}$	1.00
	FBCPred	3	$_{66}$VGSIKPDDWKAGAF$_{79}$	0.93
		4	$_{175}$GRVEYRYTQYGNKN$_{188}$	0.92
		5	$_{122}$RVGYDLNPVMPYLT$_{135}$	0.75
		6	$_{146}$NNGLDDESKFRVGWT$_{160}$	0.71
		1	$_{52}$LYLGYGWNKAKTSTVGSIKPDDWKAGAFAGWNFQQD$_{87}$	1.00
SVMTrip		2	$_{87}$DQIVYGVEGDAGYSWAKKSK$_{106}$	0.94
		3	$_{171}$DNILGRVEYRYTQYGNKNYD$_{190}$	0.65
		1	$_{86}$QDQIVYGVEGDAGYSW$_{101}$	0.94
		2	$_{158}$GWTAGAGLEAKLTDNI$_{183}$	0.93
		3	$_{50}$TGLYLGYGWNKAKTST$_{65}$	0.91
ABCpred		4	$_{136}$AGIAGSQIKLNNGLDD$_{151}$	0.91
		5	$_{176}$RVEYRYTQYGNKNYDL$_{191}$	0.89
		6	$_{65}$TVGSIKPDDWKAGAFA$_{80}$	0.88
		7	$_{114}$GFEGSLRARVGYDLNP$_{129}$	0.87
		1	$_{148}$GLDDESK$_{154}$	1.05
		2	$_{197}$RNKLDT$_{202}$	1.05
IEDB		3	$_{59}$NKAKTSTVGSIKPDDWKAG$_{77}$	1.04
		4	$_{26}$AIQEQPPVPAPVEVAPQYSWAGG$_{48}$	1.03
		5	$_{93}$VEGDAGYSWAKKSKDGLEVK$_{112}$	1.02
		6	$_{182}$TQYGNKNYDLA$_{192}$	1.02
LEPS		1	$_{28}$QEQPPVPA$_{35}$	-

Table 4 Final B-cell predicted epitopes

Number	Final B-cell epitope
1	$_{26}$AIQEQPPVPAPVEVAPQYS$_{44}$
2	$_{59}$NKAKTSTVGSIKPDDWKAGAF$_{79}$
3	$_{88}$QIVYGVEGDAGYSWAKKSKDGLEVK$_{112}$
4	$_{146}$NNGLDDESKFRVGWTAGAGLE$_{166}$
5	$_{175}$LEAKLTDNILGRVEYRYTQYGN KNYDLAGTTVRNKLDT$_{202}$

Highest score MHCI and MHCII T-cell epitopes were selected as appropriate epitopes (Table 6). The final T-cell epitopes were chosen by identifying the sequences of epitopes which were present in both MHCI and MHCII classes of T-cell epitopes (Table 6, Common color sequences were selected as final T-cell predicted epitopes).

Prediction of the secondary structure of the OMP25 protein

To assess the antigenic features of the OMP25 protein, we predicted its secondary structure using SOPMA server software. A greater proportion of extended strands and random coils present in the structure of a protein correspond with an increased likelihood of the protein forming an antigenic epitope.

The predicted secondary structure results for the OMP25 protein are shown in Figure 3. The results revealed that the proportion of random coils, β turns, α helices and extended strands (β folds) accounted for 51.64, 2.82, 20.66 and 24.88% of the secondary structure, respectively. In addition, according to secondary structure results the 48-68, 78-110, 122-128 and 188-195 regions had high random coil and extended strands, consistent with the notion that these four regions had strong antigenicity.

Prediction of the tertiary structure of the OMP25 protein

The tertiary structure of the OMP25 protein was obtained using 3DLigandSite software and compared with structure predictions obtained using VAST. The results of the predicted conformations of the epitopes are shown in Figure 4. A potential epitope located in 145-149 region (shown in blue), and the green bull is likely considered as a paratope by software.

Antigenicity of proteins

The OMP25 protein was identified as an antigen by the VaxiJen 2.0 server with a score of 0.82. The antigenicity of the final B and T-cell predicted epitopes are shown in Table 7. The results of VaxiJen 2.0 analysis indicated that two T-cell predicted epitopes (amino acids 1-10 and 14-22) were not antigenic (Table 7). According to the antigenicity ability, amino acids 154-162 were selected as a common B and T-cell epitopes.

Characterization of epitopes

The final B and T-cell predicted epitopes were illustrated in 3D structure using the 3DLigandSite server (Figure 5, A and B). The common B and T-cell predicted epitope (amino acids 154-162) is illustrated in Figure 5 (C). 3D structure analysis revealed that all predicted B and T-cell epitopes were located on the outside of the OMP25 antigen. The results of Protein Digest server analysis for determination of mass (Da), pI and enzymatic degradation site are shown in Table 8.

The development of epitope vaccines based on experimental research is very costly research with the specific needs to molecular biology and immunology technologies. With the development of bioinformatics tools, epitope prediction has become possible. The accuracy of this computational approach has been greatly enhanced using appropriate statistical methods (Sun *et al.* 2013; Chen *et al.* 2011). In this regards, several studies try to predict epitopes of desired antigen by computationally approaches and used these results with the aim of epitopic-based vaccine design (Bui *et al.* 2007; Zhang *et al.* 2010; Shen *et al.* 2010; Simon *et al.* 2010; Li *et al.* 2013; Sekhavati *et al.* 2015).

In the present study comprehensive bioinformatics analyses were conducted on the *Brucella melitensis* OMP25 antigen using online B and T-cell epitope prediction servers. We selected well-known online epitope prediction servers and a multi-method analysis approach to enhance the accuracy of epitopes prediction for OMP25 antigen. To confirm the results of our outline bioinformatics approaches (Figure 1), we first analyzed four different antigens and then the results were compared to computational outputs from experimental results.

The bioinformatics analysis of GroEL, Dnak, Omp31 and SOD antigens successfully predicted the experimentally demonstrated epitopes. Recently, Wang *et al.* (2014) experimentally demonstrated that 48-74 AA position of *Brucella melitensis* outer member protein (OMP31) is the dominant epitope and could be used as potential subunit vaccine. In this regards, this region was predicted by bioinformatics analysis to proof the applicability of the principles used in the present study (Table2).

Final prediction on B and T-cell epitope suggested 5 and 3 epitopes as antigens for B and T-cells, respectively (Table 7).

Table 5 Analysis of the MHCI and MHC II T-cell epitopes

Server	**MHC*I***	NO.	Sequence	Score
IEDB*	**A-0101**	1	$_{169}$LTDNILGRV$_{177}$	0.4
		2	$_{45}$WAGGYTGLY$_{53}$	0.6
		3	$_{181}$YTQYGNKNY$_{189}$	0.7
		4	$_{159}$WTAGAGLEA$_{167}$	1.4
		5	$_{199}$KLDTQDFRV$_{207}$	2.7
	A-0201	1	$_{199}$ KLDTQDFRV$_{207}$	0.5
		2	$_{13}$ ALLPFSATA $_{21}$	1.2
		3	$_{86}$ QDQIVYGVE $_{94}$	2.3
		4	$_{130}$ VMPYLTAGI $_{138}$	2.5
		5	$_{6}$ SLVIVSAAL $_{14}$	2.9
	B-2705	1	$_{205}$FRVGIGYKF$_{213}$	0.3
		2	$_{179}$YRYTQYGNK$_{187}$	0.4
		3	$_{119}$LRARVGYDL$_{127}$	1.1
		4	$_{155}$FRVGWTAGA$_{163}$	1.2
		5	$_{1}$MRTLKSLVI $_{9}$	2.3
Syfpeithi	**A-0201**	1	$_{6}$SLVIVSAAL$_{14}$	25
		2	$_{13}$ALLPFSATAF$_{22}$	25
		3	$_{137}$GIAGSQIKL$_{145}$	25
		4	$_{168}$KLTDNILGR$_{176}$	25
		5	$_{118}$SLRARVGYDL$_{127}$	23
	B-2705	1	$_{205}$FRVGIGYKF$_{213}$	27
		2	$_{179}$YRYTQYGNKN$_{188}$	24
		3	$_{119}$LRARVGYDLN$_{128}$	22
		4	$_{1}$MRTLKSLVIV$_{10}$	21
		5	$_{137}$GIAGSQIKLN$_{146}$	21
NetMHC	**A-0101**	1	$_{168}$KLTDNILGRV$_{177}$	0.5
		2	$_{44}$SWAGGYTGLY$_{53}$	0.5
		3	$_{180}$RYTQYGNKNY$_{189}$	0.3
		4	$_{158}$GWTAGAGLEA$_{167}$	0.2
		5	$_{198}$NKLDTQDFRV$_{207}$	0.2
	A-0201	1	$_{198}$ NKLDTQDFR $_{206}$	0.7
		2	$_{83}$ NFQQDQIVYG $_{92}$	0.7
		3	$_{12}$ AALLPFSAT $_{20}$	0.7
		4	$_{147}$ NNGLDDESKFR $_{156}$	0.6
		5	$_{167}$ AKLTDNILGR $_{176}$	0.6
	B-2705	1	$_{204}$DFRVGIGYKF$_{213}$	0.7
		2	$_{178}$EYRYTQYGN$_{187}$	0.6
		3	$_{118}$SLRARVGYDL$_{127}$	0.5
		4	$_{154}$KFRVGWTAG$_{162}$	0.4
		5	$_{1}$MRTLKSLVIV$_{10}$	0.3
NetCTL	**A-0201**	1	$_{204}$DFRVGIGYK$_{212}$	1.2
		2	$_{131}$MPYLTAGIA$_{139}$	1.1
		3	$_{13}$ALLPFSATA$_{21}$	1.1
		4	$_{85}$QQDQIVYGV$_{93}$	0.9
		5	$_{2}$RTLKSLVIV$_{10}$	0.8
Propred1	**B-2705**	1	$_{179}$YRYTQYGNKN$_{188}$	9
		2	$_{119}$LRARVGYDLN$_{128}$	7
		3	$_{205}$FRVGIGYKF$_{213}$	7
		4	$_{1}$MRTLKSLVIV$_{10}$	6
	MHC*II*			
IEDB*	**DRB1-0101**	1	$_{1}$MRTLKSLVIVSAALL$_{15}$	0.14
		2	$_{4}$LKSLVIVSAALLPFS$_{18}$	0.14
		3	$_{2}$RTLKSLVIVSAALLP$_{16}$	0.28
		4	$_{3}$TLKSLVIVSAALLPF$_{17}$	0.28
		5	$_{5}$KSLVIVSAALLPFSA$_{19}$	0.28
	DRB1-0401	1	$_{1}$MRTLKSLVIVSAALL$_{15}$	0.7
		2	$_{52}$LYLGYGWNKAKTSTV$_{66}$	2.5
		3	$_{53}$YLGYGWNKAKTSTVG$_{67}$	2.5
		4	$_{54}$LGYGWNKAKTSTVGS$_{68}$	2.5
		5	$_{55}$GYGWNKAKTSTVGSI$_{69}$	2.5
Propred	**DRB1-0101**	1	$_{6}$SLVIVSAALL$_{15}$	3
		2	$_{1}$MRTLKSLVIV$_{10}$	2.5
		3	$_{204}$DFRVGIGYKF$_{213}$	1.8
		4	$_{154}$KFRVGWTAGA$_{163}$	0.8
		5	$_{126}$DLNPVMPYLTA$_{135}$	0.2
	DRB1-0401	1	$_{1}$MRTLKSLVIV$_{10}$	3.5
		2	$_{154}$KFRVGWTAGA$_{163}$	3
		3	$_{14}$LLPFSATAFA$_{23}$	2
		4	$_{122}$RVGYDLNPVMP$_{132}$	2
		5	$_{21}$AFAADAIQEQ$_{30}$	1.8

* Low score= good binders.

Table 6 Final T-cell epitopes after filtration

		Final T-cell epitopes with highest score
MHC*I*	**A-0101**	$_{45}$WAGGYTGLY$_{53}$ $_{159}$WTAGAGLEA$_{167}$ $_{169}$LTDNILGRV$_{177}$ $_{180}$RYTQYGNKNY$_{189}$
	A-0201	$_{13}$ALLPFSATAF$_{22}$ $_{85}$DQIVYGV$_{93}$ $_{130}$VMPYLTAGIAGSQIKL$_{145}$ $_{198}$NKLDTQDFRV$_{207}$
	B-2705	$_{1}$MRTLKSLVIV$_{10}$ $_{118}$SLRARVGYDLN$_{128}$ $_{154}$KFRVGWTAGA$_{163}$ $_{178}$EYRYTQYGNKN$_{188}$ $_{204}$DFRVGIGYKF$_{213}$
MHC*II*	**DRB1-0101 and DRB1-0401**	$_{1}$MRTLKSLVIV$_{10}$ $_{14}$LLPFSATAFA$_{23}$ $_{122}$RVGYDLNPVMP$_{132}$ $_{154}$KFRVGWTAGA$_{163}$ $_{204}$DFRVGIGYKF$_{213}$

* Common color sequences were selected as final T-cell predicted epitopes.

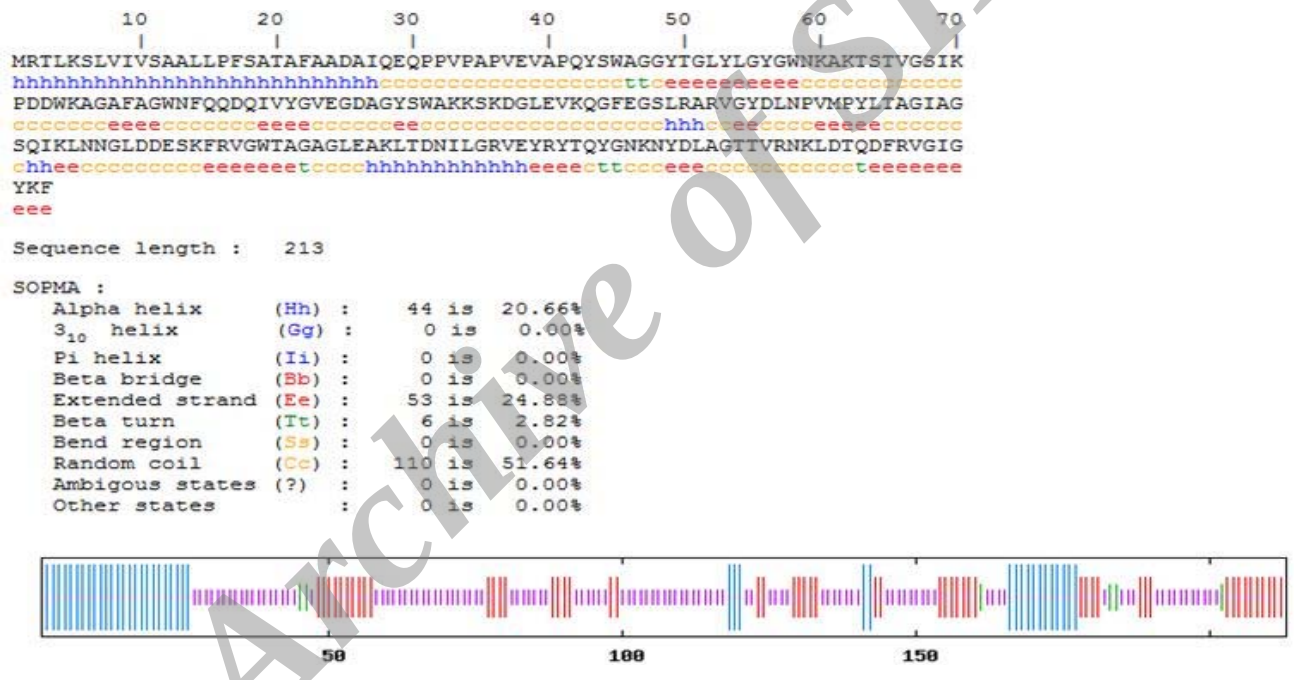

Figure 3 Secondary structure prediction results for the OMP25 protein. An increased number of extended strands and random coils in the protein corresponded with an increased likelihood of the protein forming an antigenic epitope. Lines in different colors represent different secondary structures: Blue: α helix; Green: β turn; Red: extended strand and Purple: random coil

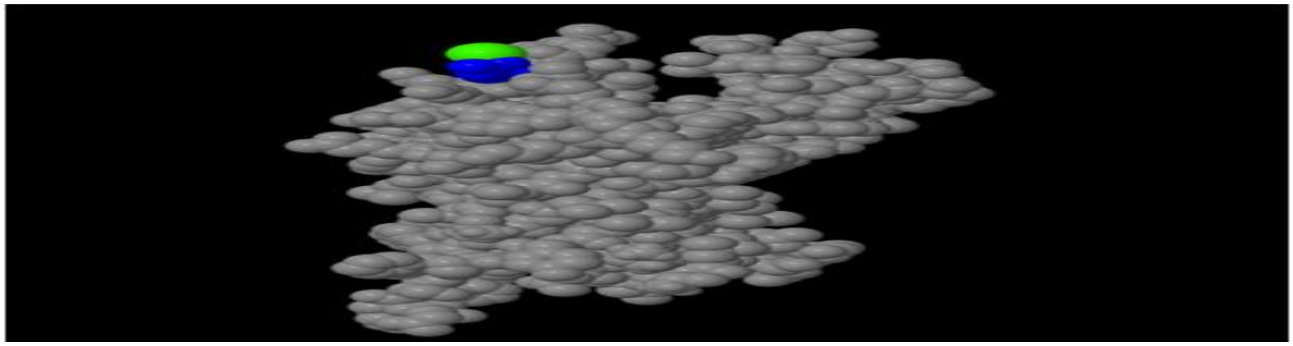

Figure 4 Tertiary structure prediction results for the OMP25 protein. Blue and green colors are the proposed epitope (145-149 region) and paratope, respectively

Table 7 The antigenicity ability of predicted epitopes

Number	Final B-cell epitope	VaxiJen score	Final T-cell epitope	VaxiJen score
1	$_{26}$AIQEQPPVPAPVEVAPQYS$_{44}$	0.66	$_{1}$MRTLKSLVIV$_{10}$	-0.1*
2	$_{59}$NKAKTSTVGSIKPDDWKAGAF$_{79}$	0.7	$_{14}$LLPFSATAF$_{22}$	0.23*
3	$_{88}$QIVYGVEGDAGYSWAKKSKDGLEVK$_{112}$	1.2	$_{122}$RVGYDLNPVMPY$_{132}$	0.7
4	$_{146}$NNGLDDESKFRVGWTAGAGLE$_{166}$	1.1	$_{154}$KFRVGWTAG$_{162}$	1.38
5	$_{175}$LEAKLTDNILGRVEYRYTQYGN KNYDLAGTTVRNKLDT$_{202}$	0.92	$_{206}$RVGIGYKF$_{213}$	1.8

* Probable non-antigen.

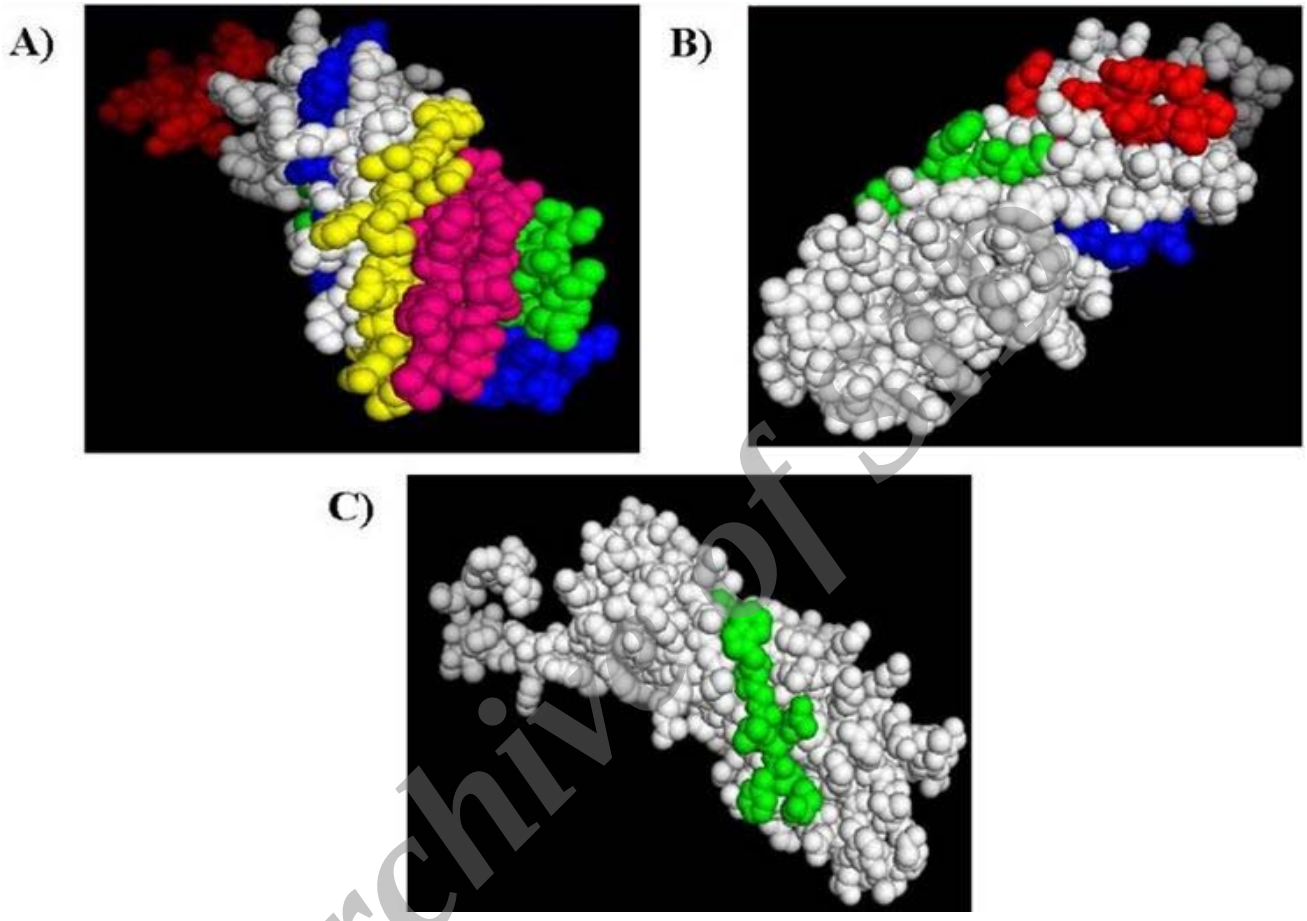

Figure 5 B-cell, T-cell and common predicted epitopes. (A): B-cell epitopes: 26-44, 59-79, 88-112, 146-166 and 175-202 were shown by red, green, blue, yellow and pink color, respectively. (B): T-cell epitopes: 122-132, 154-162 and 206-213 were identified by red, green and blue, respectively. (C): The same epitope between B- and T-cell epitopes: 154-162 was identified by green

Table 8 Protein digest analysis of final B-and T-cell epitopes

B-cell epitopes	Mass (Da)	pI	Undigested enzyme
AIQEQPPVPAPVEVAPQYS	2020.27	3.8	Trypsin, Clostripain, Cyanogen Bromide, Iodose Benzoate, AspN, Trypsin K, Trypsin R
NKAKTSTVGSIKPDDWKAGAF	2221.5	9.5	Clostripain, Cyanogen Bromide, Staph Protease, Trypsin R
QIVYGVEGDAGYSWAKKSKDGLEVK	2728.05	6.2	Trypsin R, ProlineEndopept, Cyanogen Bromide, Clostri-pain
NNGLDDESKFRVGWTAGAGLE	2236.38	4.54	Cyanogen Bromide, ProlineEndopept
LEAKLTDNGRVEYRYTQYGNKNYD-LAGTTVRNKLDT	4407.91	8.3	ProlineEndopept, Iodose Benzoate, Cyanogen Bromide
T-cell epitopes	**Mass (Da)**	**pI**	**Undigested enzyme**
RVGYDLNPVMPY	2423.65	5.83	Trypsin K, Iodose Benzoate, Staph Protease
KFRVGWTAG	1021.19	11	AspN, Staph Protease, ProlineEndopept, Cyanogen Bromide
RVGIGYKF	939.13	9.9	Cyanogen Bromide, ProlineEndopept, Iodose Benzoate, AspN, Staph Protease

Our results further showed that amino acids 154-162 residues could be a common epitope with the ability to stimulate both cell-mediated and humoral immunity system. The results of secondary and tertiary analysis showed that the common predicted B and T-cell epitope was located in the random coil regions on the surface structure of OMP25 antigen. Random coil regions are located on the surface of the protein, where it is necessary for the surface structure to make appropriate binding to ligands, and have a high possibility of forming epitopes (Li *et al.* 2013). To prevent degradation of peptide during antigen processing, epitope should lack proteosomal recognition site (Toes *et al.* 2001). Accordingly predicted B and T-cell epitopes were analyzed for enzymatic degradation sites (Table 8). There were no recognition sites for AspN, Staph Protease, Proline Endopept and Cyanogen Bromide enzymes which are the central enzymes responsible for protein degradation. *In vitro* synthesis of peptides and experimental testing are essential to determine the predicted epitopes as a part of an effective vaccine against *Brucella* species. To do this, our laboratory has focused their researches in this direction.

CONCLUSION

In the present study, a wide range of on-line prediction software was used to predict B and T-cells epitopes, secondary and tertiary structure and antigenicity of *Brucella* OMP25 antigens. Bioinformatics analysis showed a common B and T-cell epitope was identified at 154-162 amino acid position. Therefore, this region has proper epitope characterization and so may be useful for producing recombinant vaccine. However, *in vitro* study is necessary for these results.

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