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Research Article
In silico Design, Synthesis and Potency of an Epitope-based Vaccine Against Foot-and-mouth Disease Virus

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Abstract

Objective: Foot-and-mouth disease virus (FMDV) is an acute and highly contagious virus that infects cloven-hoofed domesticated ruminants. Discovery of effective T-helper (Th) epitopes is critical for the formation of stable interactions with Major Histocompatibility Complex (MHC) molecules and the induction of an adaptive immune response specific to the pathogen. **Methodology:** In this study, we sought to discover potential Th peptides that more broadly recognize supertypes of bovine leukocyte antigen (BoLA) using immuno-informatics approaches, including the IEDB database and ProPred tools. The peptide VP1Th was used to generate a combinatorial peptide library, VP1Thcomb, which mimicked population-spanning. **Results:** Th epitopes based on the substitution of amino acids at optimal anchors for peptide-MHC interactions that exhibited diverse binding pockets in the 130 polymorphic BoLA-DR3 alleles. Additionally, the primary and auxiliary anchor residues were improved to increase the ligand affinity of binding MHC molecules by substituting arginine at anchor residue 2 and to extend the peptide length at both ends of the Th epitope binding core region, respectively. Finally, the epitope-based vaccine VP1Thcomb-FMDV was chemically synthesized and vaccine potency reached 12.51 and 8.05 bovine PD_{50} per single dose administration challenged with the two serotypes of 10,000 BID_{50} FMDV, Asia 1 and O, respectively. **Conclusion:** Strong associations were also observed between virus neutralization test titers and protection. In conclusion, the strategy of epitope-based vaccine development provided a minimal structure of well-defined antigen to stimulate protective immunity against FMDV.

Key words: Epitope-base vaccine, FMDV, Th epitope, MHC, combinatorial peptide library, homology modeling, VP1, BoLA-DR3, anchor residues, vaccine potency

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Foot-and-mouth disease virus (FMDV) is an acute and highly contagious virus that causes economic loss in susceptible cloven-hoofed animals. Foot-and-mouth disease (FMD) has the potential to have a severe impact on international free trade in susceptible animals and animal products. The FMD can be controlled by slaughter or by regular vaccination[1]. The slaughter of infected, recovered and FMD-susceptible animals has been preferred for the sanitary treatment of herds that are otherwise free of the disease because under the current regulations of the Office International des Epizooties (OIE), the presence of vaccinated animals results in loss of disease-free status, influencing the sale of livestock[2].

An epitope can be defined as an antigenic determinant and is a group of amino acids derived from a protein antigen that is recognized by the immune system[3]. A major challenge in the development of epitope-based vaccines is that they are based on the selection of epitope peptides from widely varying pathogen genomes and rely on the diversity of the host cellular immune system to optimize an immune response for a particular pathogenic population[4].

Based on available knowledge of epitope-base immunity, a synthetic chimeric peptide that encodes B- and T-epitopes against FMDV was developed[5]. Earlier investigations of FMDV pursued recognition of one of the major antigenic sites on the FMDV capsid protein, VP1, including the surface-exposed G-H loop (amino acids positions 140-160) characterized as a primary B-epitope[6]. Therefore, incorporation of T-helper (Th) epitopes is not sufficient for the effective design of epitope-based vaccines, the selected peptides should effectively cover strain population and the Th epitope peptides must interact with various Major Histocompatibility Complex (MHC) alleles in cattle[7]. Based on the immune epitope database analysis resource (IEDB-AR)[8] and certain properties of pocket profiles and anchor residues from the bovine MHC-peptide modeling structures[9], epitope-engineering[10] was applied to identify potential bovine Th epitopes that would elicit an optimal immune response and could serve as epitope-based vaccines against FMDV.

MATERIALS AND METHODS

An outline of the methodology undertaken for Th peptide VP1Th selection and for combinatorial peptide library VP1Thcomb design was portrayed in Fig. 1a.

T-epitope VP1Th peptide selection: The prominent G-H loop of the VP1 capsid of FMDV, including a surface-exposed highly conserved Arg-Gly-Asp (RGD) tripeptide has been identified as a major B-cell epitope for Neutralizing Antibodies (NAs). However, administering G-H loop synthetic peptides alone resulted in limited induction of NAs or ineffective protection due to a lack of T-epitope for promiscuous recognition by cattle MHC alleles[11].

In Table 1, we present potential Th peptides for recognition of additional supertypes of bovine leukocyte antigen (BoLA) by using immuno-informatics approaches including the IEDB database and ProPred tools. The selected peptide, VP1Th, bound to MHC using the T-cell response and MHC ligand binding analysis tools[12] available at the IEDB-AR (www.iedb.org). Allele-specific consensus percentile ranks of all algorithms queried by IEDB tools were utilized. The amphipathic helix calculation helical wheel projection was carried out with the EMBOSS[13] software package according to Eisenberg' scales for potential Th epitopes[14].

3D modeling for the VP1Th and BoLA-DR complex: Initial 3D models of the epitope peptide VP1Th and BoLA-DR3 molecules were created according to the homology modeling strategy, which ensures the 9-mer peptide from FMDV VP1[15] (PDB code: 1ZBA, chain 1) and human MHC HLA-DR structures[16] (PDB code: 1D5M, chain A and B) as a backbone template using the MODELLER module[17], respectively. A program setting of 4.0 Å as root mean square deviation among initial model and by full model optimization was used. We predicted the binding free energies of epitope peptide to macromolecular BoLA-DR3 protein by receptor-ligand docking simulation software based on the Lamarckian genetic algorithm, AutoDock[18] (version 4.2). To select the best model, we used ANOLEA and PROCHECK in SWISS-MODEL[19] by maintaining the default analysis parameter to evaluate the local model quality and stereo-chemical packing quality, respectively.

Binding pocket profiles evaluation: A total of 130 sequences of BoLA-DR3 α chain and β chain alleles were BoLA-DR3*0101 from the IPD-MHC database[20]. Multiple alignments of BoLA-DR3 sequences with the modeling 3D structures were performed to link polymorphic suitable residues to given pockets (Fig. 2a). Table 2 displays pocket profiles of BoLA-DR3 and demonstrated 20 BoLA-DR3 alleles that were assigned to pockets of the original 130 BoLA-DR3 alleles according to their classification[9]. Pockets on different alleles sharing the same

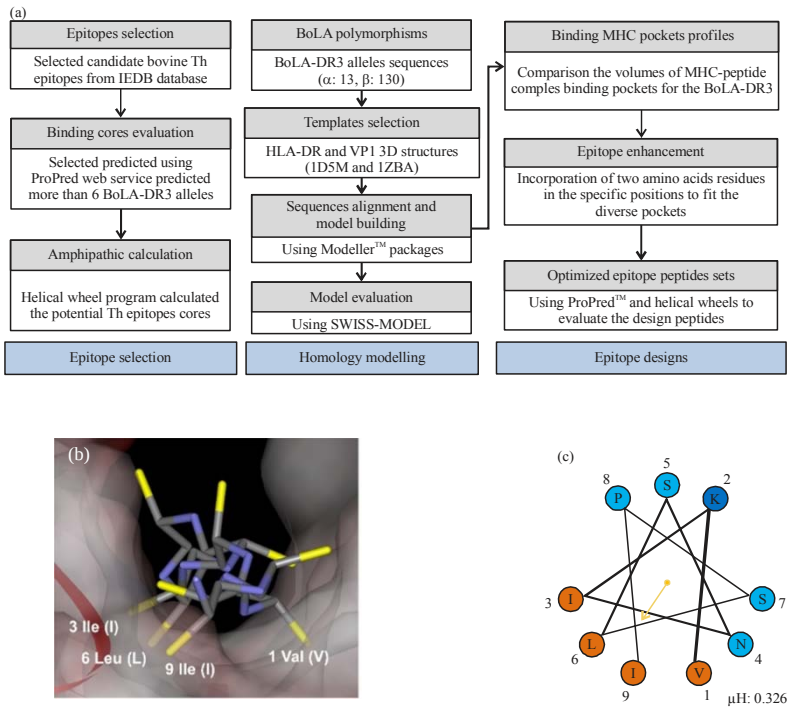


Fig. 1(a-c): Bovine Th epitope-based vaccines selection and designs roadmap, (a) The workflow of *in silico* selection and design of bovine Th epitopes. The design strategy was separated into three stages including epitope selection, homology modeling and epitope designs, (b) A diagram of the helical peptide towards in the MHC class II binding pockets. The pockets are viewed in the plane of the peptide binding and the molecular surface of the HLA-DR1 (PDB ID: 1d5m), shown in blue, with the Cα trace of influenza virus peptide. Side chains of residues are shown as yellow sticks in surrounding views and (c) The helical wheel represent the amphipathic peptide-binding core and rotational angle is approximately 130°C. Side chains of residues 1, 4, 6 and 9 of Th epitopes pointed toward the corresponding binding pockets that were revealed at the bottom of MHC binding clefts. The colored circles indicated the hydrophobic, uncharged polar, positive charged residues illustrated as red, blue and deep blue, respectively. The yellow arrow indicated the magnitude and orientation of hydrophobic moment (μH)

Table 1: Representative the antigenicity of selected peptide used in this study for bovine epitope-based vaccines design

Selected peptides	Source[a]	Helical hydrophobic moment[b]	No. of binding/tested MHCII alleles[c]	Epitope ID[d]	Assays[d]	Epitope core[e]
					T-cell response Positive: 1 Positive-low: 1	
	FMD virus				B-cell response	
				27470		
VP1Th	VP1 protein (32-50)	0.326	12/51		Positive: 6	VDFIMDRFVKINSLSPTHV
				31373		
					MHC ligand binding Positive: 3 Positive-high: 2 Positive-intermediate: 1	

[a]The FMDV virus capsid protein 1D from GenBank protein accession: AHY00720, [b]The evaluation of amphipathic helical wheel are greater than 0.3 hydrophobic moment (μH) calculated according to Eisenberg's method, [c]The server can predict the binding of peptide to 51 HLA-DR alleles, [d]The epitope ID that a unique primary identifier and the results from the different category of assays are used the linear epitope searching as 90% blasting homology in IEDB database (v.2.13.1), [e]The core of promiscuous Th cell epitope (underlined) that is 9 amino acids in length predicted by ProPred web server

polymorphic residues exhibited similar pocket specificity profiles. To analyze the relationship between the binding pocket profiles of BoLA-DR3 and the geometry of their binding pockets, we used the PocketPicker[21] algorithm that translated

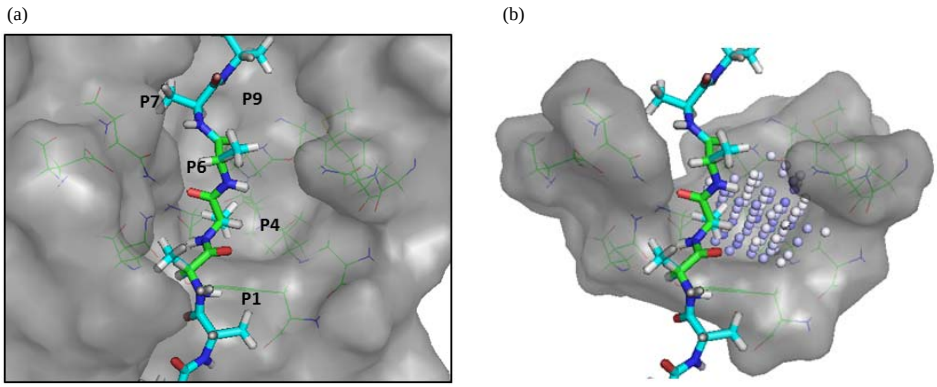


Fig. 2(a-b): Pockets in the BoLA-DR3*0101 peptide binding site and pocket volume estimated, (a) Top view and of the molecular surface (Soft style render by ViewerLiteTM 4.2) of the BoLA-DR3*0101 peptide-binding site, shown in gray with the surrounding amino acids shown in thin green lines. And the peptides represented as stick model with the side chain, carbon atoms are green, nitrogen atom blue, nitrogen atom gray and oxygen atoms red. Pocket 4 that accommodate peptide side chains in detail in surrounding views and are numbered according to their distance along the peptide from the most prominent labelled as P1, 4, 6, 7 and 9 and (b) The shape of packet conformation induced by BoLA-DR3*0101 and potential binding site are detected by grid-based PocketPicker represented with darker spheres indicating grater buriedness. The grid probes revealed rectangular grid with 1 Å mesh size closely above the protein surface

Table 2: Construction of BoLA-DR3 virtual pocket profile matrices using assigned pocket

Selected alleles[†]	Profiles[‡]
BoLA-DRB3*0101	1;1;1;1;1
BoLA-DRB3*0301	2;3;2;4;2
BoLA-DRB3*03021	2;3;2;4;2
BoLA-DRB3*0501	2;4;3;5;3
BoLA-DRB3*0503	1;4;1;5;3
BoLA-DRB3*0504	1;4;4;5;3
BoLA-DRB3*0701	2;6;2;7;4
BoLA-DRB3*1802	2;6;4;9;7
BoLA-DRB3*1902	2;4;1;5;2
BoLA-DRB3*2002	1;6;2;9;6
BoLA-DRB3*2003	1;6;2;8;8
BoLA-DRB3*2401	1;5;2;9;5
BoLA-DRB3*2701	1;5;4;7;2
BoLA-DRB3*2702	3;5;4;2;1
BoLA-DRB3*2707	2;5;4;7;1
BoLA-DRB3*3601	2;6;1;9;8
BoLA-DRB3*4001	2;6;1;1;3
BoLA-DRB3*4303	3;4;2;6;4
BoLA-DRB3*4802	2;6;2;9;1
BoLA-DRB3*6401	2;6;4;9;3

[†]The selected BoLA-DR3 alleles indicated these alleles used to construct 3D structures by homology modelling detail procedure shown in material and methods, [‡]Virtual BoLA-DR3 matrices were assembled according to the modular structure of the BoLA-DR3 clefts as revealed in Table 3. Profiles for pockets 1, 4, 6, 7 and 9 were derived from the IPD-MHC database. For relative peptide position 1, only aliphatic (Ile, Leu, Met, Val) and aromatic (Phe, Trp, Tyr) amino acid residues are fitted into this pocket, for BoLA-DR3 alleles with a b86 residue composing pocket 1. The 1st number indicated whether the allele has a Gly (=1) or a Val (=2) or a Met (-3) at β 86 position (Table 3). The 2nd number represents the identification number of the pocket 4 profile. The 3rd, 4th and 5th number indicates the identification number of the pocket 6, 7 and 9 profiles, respectively

the shape and degree of buriedness of potential binding pockets into autocorrelation vectors. One grid probe within the cubic grid defines a volume of 1 $Å^3$ (Fig. 2b).

V1Thcomb design and synthesis: Figure 3 presents the design strategies for the combinatorial peptide library, VP1Thcomb from the VP1Th peptide that included the incorporation preference for amino acids at the necessary positions for efficient peptides-MHC interactions and improved the primary and secondary anchor residues to provide additional affinity for MHC molecules binding to a combinatorial peptide library (VP1Thcomb) that mimics the population-spanning Th epitopes. The VP1Thcomb-FMDV peptide library was synthesized using an applied biosystems peptide synthesizer. Completed peptides were cleaved from the solid support and side chain protecting groups using 90% trifluoroacetic acid. Synthetic peptide preparation was characterized for correct composition by matrix-assisted laser desorption time-of-flight mass spectrometry and by reverse phase HPLC. The combinatorial peptides library for vaccine was characterized by size exclusion chromatography to a specification that required 90% of the integrated area to exceed a mass threshold limit value and by Edman degradation for N-terminal amino acid analysis.

Vaccination challenge and potency: Vaccine potency is estimated in vaccinated animals directly and PD_{50}

Fig. 3: VP1Thcomb-FMDV amino acid sequence of the epitope-based vaccines design against FMDV in this study. Th epitope used the combinatorial library VP1Thcomb site shown. The G-H loop region of the VP1 capsid protein was identified as the primary immunogenic site for neutralizing antibodies. The linker between the Th epitope and B-epitope of the G-H loop region VP1 was an ε-Lysine. The surface exposed RGD tripeptide (underlined) is highlighted. The heteroclitic peptides P1, P2 and P9 indicated the positions corresponding to the MHCII binding pockets 1, 2 and 9 predicted by the ProPred web server, respectively

(50% protective dose) values were examined to assess the epitope-based peptide vaccine potency described by the OIE Reference Laboratory[2]. Three groups of five 5-6-month-old cattle were included and received either 1 mL full dose, 1/3 dose or 1/9 dose. Each animal was immunized one time with epitope-based vaccines peptides with montanide ISA 51 adjuvant at a 1:1 ratio (v/v) for 28 days by parental injection at the ear. In addition, two cattle were immunized with sterilized PBS buffer as a negative control. All cattle were challenged with 10,000 BID_{50} (50% bovine infectious does) of stereotype Asia 1/O by subcutaneous injection after 28 days of immunization. All experimental cattle (approximately 6 months old Holstein steers) were obtained from a certified vendor. Animals were monitored daily throughout the entire study periods for symptoms, including body temperature, mouth pathology and feeding habits for ten days and the bovine PD_{50} value was calculated. At the experimental endpoint, we used humane intravenous injection of an overdose of phenobarbital (90 mg kg^{-1}) following sedation by intramuscular injection of xylazine hydrochloride for all animals. The bovine PD_{50} values were calculated by the Reed-Muench method[22]. All experiments involving the use of bovine tissue samples and vaccination protocols were approved by the institutional review board of the CSMU committee on biospecimen unitization (Chung Shan Medical University Hospital Institutional Review Board, CSMU-101-25).

Assays for anti-FMDV Virus Neutralization Test (VNT): The anti-FMDV VNT assays were described in the OIE terrestrial manual (Chapter 2.1.5). Cattle sera were collected at 7, 14 and 28 days post vaccination. Sera from tested animals were inactivated at 56°C for 30 min. The serum from 10 cattle were added (1×10^4 $TCID_{50}$ O type and Asia 1 type FMDV) for ninety min at 37°C into 96 well flat-bottomed plates. After the incubation 50 µL of 3×10^4 BHK21 cells suspended in Dulbecco Modified Eagle Medium (DMEM) containing 5% fetal bovine serum were added into each plate for 48 h at 37°C 5% CO_2. Titers were expressed as the final dilution of the serum from the serum/virus mixture where 50% of the cells were protected. The positive standard serum was within two-folds of its expected titer.

RESULTS

Selected VP1Th epitope peptide characteristics: A survey of the targeted VP1Th peptide from the IEDB-AR database, which is the most likely antigenic determinant from FMDV VP1 amino acids in positions 32-50 was conducted on a set of Th peptides from effective antigenic epitopes in cattle[23] to support the design of epitope-based vaccines (Table 1). Additionally, the immunogenicity characteristics of the VP1Th peptide were evaluated using various categories of assays including one T-cell response, six B-cell responses and three MHC ligand binding assays recorded as epitope ID 27470 and 31373 in the IEDB database[12]. Effective MHC-bound peptides must have a strong helical structure with hydrophobic and hydrophilic zones on the opposite side of the helix (Fig. 1b). To identify the core epitopes of VP1Th peptide involved in the amphipathic α-helices, 0.326 hydrophobic moments were calculated by helical wheel analysis (Fig. 1c). Notably, two meta-analyses of the VP1Th peptide have verified T-cell binding activities from the different linear epitope sequences[24].

Binding affinity profiles of BoLA-DR3 alleles: The coding sequences of BoLA-DRA is monomorphic and the BoLA-DRB3 gene has more than 130 identified alleles[25]. The MHC peptide-binding groove has various chemically distinct binding pockets (termed 1-9). Table 3 reveals that most of the polymorphic residues on the binding pockets 1, 4, 6, 7 and 9 that coming from the BoLA-DR3 allele's b-chain, which aligned with multiple BoLA-DR3 sequences[9]. Twenty molecular docking modeling structures of bovine MHC-peptide complex facilitated the identification of alleles with similar structural features and/or peptide specificities because MHC allelic variants generally bound to a distinct set of epitope peptides (Table 3).

Table 3: BoLA-DR3 independent of pocket profiles leads to wide coverage the DR binding cleft specificity

Pockets	Profile ID[#]	Residues (BoLA-DR3 β chain)
1	1	86G
	2	86V
	3	86M
4	1	13R;70R;71A;74A;78Y
	2	13S;70R;71A;74A;78Y
	3	13K;70R;71A;74A;78Y
	4	13S;70E;71R;74E;78V
	5	13S;70E;71R;74N;78V
	6	13S;70R;71R;74Y;78Y
6	1	11S
	2	11Y
	3	11T
	4	11H
7	1	28D;30Y;47Y;61C;67F;71A
	2	28D;30Y;47Y;61W;67I;71A
	3	28N;30Y;47Y;61W;67I;71A
	4	28E;30S;47Y;61W;67I;71R
	5	28D;30R;47Y;61L;67F;71E
	6	28D;30H;47F;61W;67F;71R
	7	28D;30C;47F;61W;67F;71R
	8	28D;30C;47F;61W;67I;71R
	9	28D;30Y;47F;61W;67F;71K
9	1	9E;37Y;57V;60Y;61C
	2	9E;37F;57D;60Y;61W
	3	9E;37N;57D;60Y;61W
	4	9E;37F;57V;60Q;61L
	5	9E;37F;57S;60Q;61L
	6	9Q;37Y;57S;60Y;61W
	7	9E;37T;57D;60Y;61W
	8	9E;37T;57S;60Y;61W

[#]Based on the polymorphic residues positions by *Sturniolo et al.*[9] binding pockets 1, 4, 6, 7 and 9 coming from the BoLA-DR3 alleles are mainly form by DR β chain polymorphic residues and are responsible for the allele specificity of BoLA-DR3-ligand interaction are assigned in the corresponding positions

The proposed the binding pockets of various BoLA-DR3 alleles had distinct electrostatic complementarity and sizes with proximal MHC residues[26]. For each pocket, contact residues were defined as BoLA-DR3 specific contacts derived from the mean pocket size and degree of buriedness of the sets of extracted pockets (Fig. 4b). Pocket profiles were occupied and bound by 20 different amino acid side chains of Th epitope in a manner that depended on the volumes, degree of buriedness and binding affinities of bovine MHC molecules for scoring the favored amino acids in bovine epitope prediction (Fig. 4c). For instance, the pocket 1 in the twenty selected models derived from 130 BoLA-DR3 alleles to be unoccupied cavities with a wide range of volumes from 50-195 $Å^3$ (Fig. 4a). The favored amino acids that should be preferentially incorporated at pockets 1 and 9 are required for improving the interactions between Th epitope and bovine MHC molecules in the diverse BoLA-DR3 alleles used for population-spanning designs. Additionally, to generate the relative binding value (rbv) of corresponding amino acids to four independent binding pockets showed higher frequency profiles that included pocket 1, 6, 7 and 9 (Fig. 4c). These pockets represented the majority of BoLA-DR3 peptide binding performance[9]. The roles of the amino acids were found to be as binding anchors and for specific interactions with pocket 1 and 9. In pocket 1, profile 1 favors aromatic amino acids (F; rbv: 1.62) and profile 2 prefers aliphatic amino acids (V; rbv: 1.65). In pocket 9, profile 8 favors amino acids (T; rbv: 1.32) and profile 3 prefers aliphatic amino acids (I; rbv: 1.36) (Fig. 4c).

Epitope-based vaccine VP1Thcomb-FMDV designs: In generally, numerous anchor residues of Th epitope peptides are modified for enhancement by substituting the amino acids in the natural sequence that act as weak primary MHC anchors for optimal anchors. VP1Thcomb, a combinatorial peptide library derived from the original peptide VP1Th, was optimized by actual identification of VP1Th from IEDB-AR resources. Here, combinatorial epitope-based vaccines peptides (VP1Thcomb-FMDV) that provided a mixture of desired amino acids at corresponding pockets 1 and 9 contributed significantly to the binding energy and were required for efficient peptide binding in diverse bovine MHC alleles (Fig. 3).

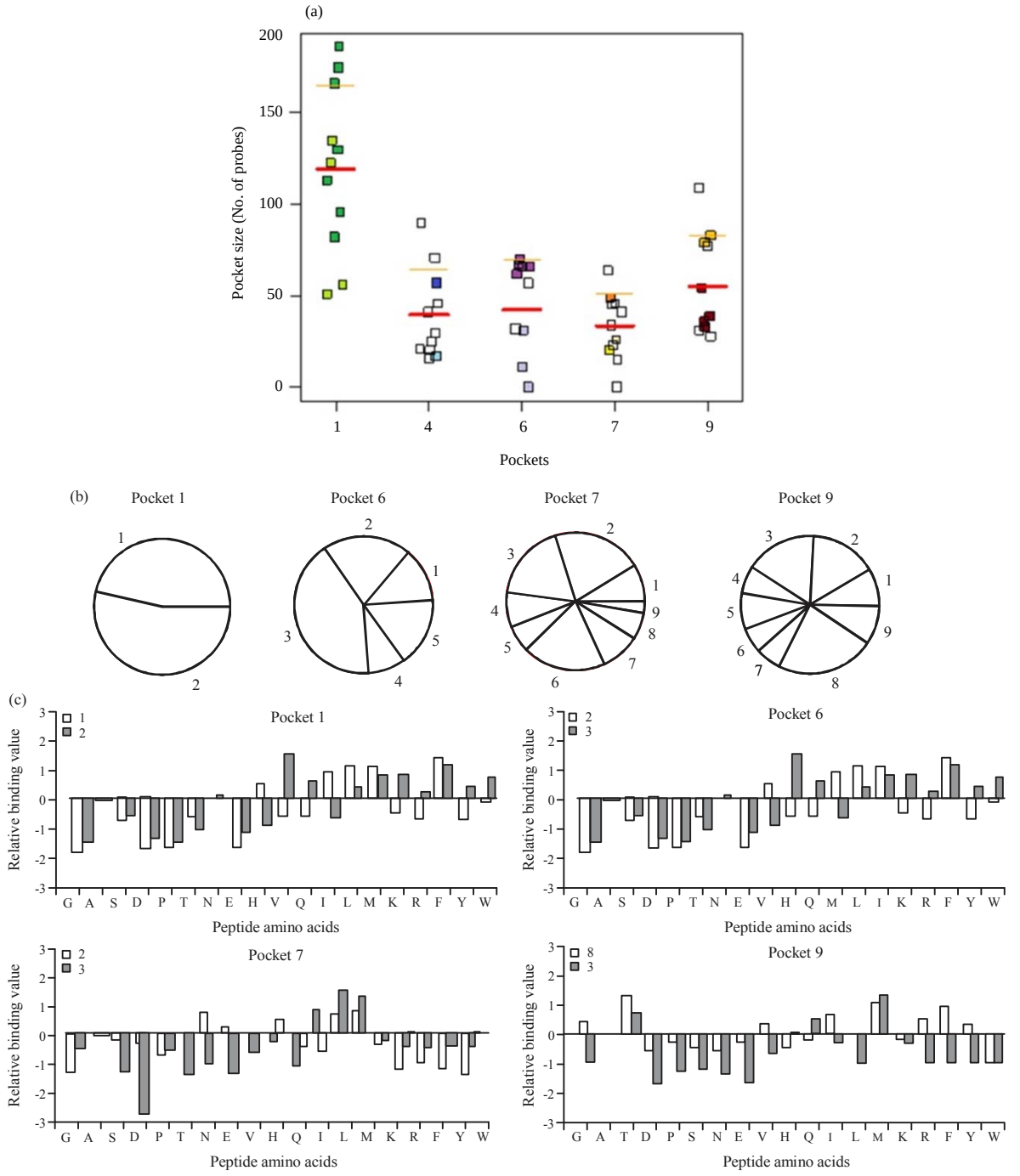
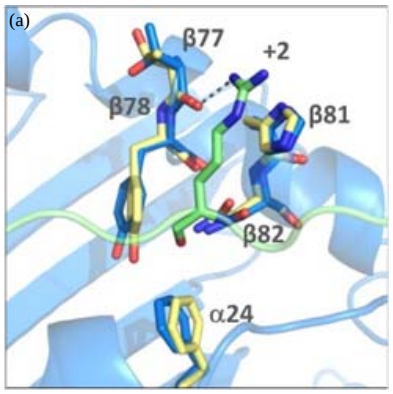


Fig. 4(a-c): Distribution of pockets volumes and profiles in the peptide binding sites from 130 BoLA-DR3 alleles, (a) The pockets volume formed by the peptide binding groove of 20 categories of BoLA-DR3 homology models which alleles proposed by IPD database. Pocket sizes were estimated by a grid-based technique, one grid probe defined a cubic grid of 1 $Å^3$ calculated by the PocketPicker. The 1, 6, 7 and 9 pockets are represented along the x-axis separately. The horizontal lines reveal the mean of packet volumes and are shown on the y-axis, (b) Colored dots corresponding to the profiles in Pie charts indicated the frequency distribution of polymorphic residues exhibit a pocket specificity profile among 20 categories of 130 BoLA-DRB3 alleles described from the IPD database. Top 2 higher frequency profiles are colored and numbers are represented as profile identifier and (c) The relative affinities of amino acids for individual BoLA-DR3 alleles represented by *Sturniolo et al.*[9] are only shown as higher frequency profiles in (b). Relative binding values (rbv; y-axis) were calculated by the logarithm of normalizing experimental IC_{50} data

(a)



(b)

Polymorphic residues of pocket 2 from BoLA-DRB3	Conserved residue	Frequency in BoLA-DRB3 alleles
β57	Asp	80/130
β77	Thr	94/130
β78	Tyr	86/130
β81	His	121/130
β82	Asn	130/130

Fig. 5(a-b): Schematic representation of the modular structure of the BoLA-DR3 binding pocket 2. Conserved residues form hydrogen bonds or contacts with the HLA-DR ligands in MHC-peptide interactions, (a) Conserved residues of HLA-DR and BoLA-DR3 interacted (<4 Å) with Arg (position +2 of Th epitope cell peptide) are α24, β77, β78, β81 and β82. Carboxyl oxygen of β77 forms a hydrogen bond with one side chain nitrogen of Arg. Blue ribbons and sticks are HLA-DR structure PDB ID-1qad and binding peptide are colored as green stick. Yellow sticks represent corresponding residues of BoLA-DRB3*0101 constructed using homology modeling and are superimposed based on human HLA structure and (b) The frequencies of conserved residues occurrence in BoLA-DRB3 alleles are from the IPD. The conserved residues are from the reference allele BoLA-DRB3*0101

Additional anchor residue in binding peptides at position P2 across the binding cleft were additive and enabled short peptides to bind tightly to MHC molecules[27]. The contacted residues, β57 and β77-β82, formed a hydrogen bond with the main-chain oxygen in MHC-peptide complex models as presented in Fig. 5a. These contacted residues Asp57, Thr77, Tyr78, His81 and Asn82 for the anchor position P2 also determined the highly frequencies of 80, 94, 86, 121 and 130 in the 130 BoLA-DRB3 alleles, respectively. The suboptimal residue at anchor position P2 in the binding core of VP1Thcomb epitope peptides increased the binding affinity for bovine MHC molecules (Fig. 5b).

Potency of VP1Thcomb-FMDV epitope-based vaccines: The results herein demonstrated the potency of the epitope-based vaccines (VP1Thcomb-FMDV) peptide library (Fig. 3) with 3 weeks vaccinations reaching 12.51 and 8.06 PD_{50} per single administrated dose for FMDV serotypes Asia 1 and O, respectively (Table 4). Emergency vaccines are generally required to have a minimum potency PD_{50} of 6, whereas vaccines for prophylactic use are commonly formulated to at least potency of 3 PD_{50}. This study showed that the epitope-based vaccines may be more efficacious against FMDV and serotype Asia 1 than against serotype O, under a challenge by a 10,000 BID_{50} dose.

FMDV-specific virus neutralization test: Immunized cattle underwent VNT to measure the titer of neutralizing antibodies (NA)s 14, 21 and 28 days post vaccination (dpv) via intramuscular inoculation at a site in the neck, in response to challenges with 4 and 6 serotypes of O or Asia 1 FMDV (6-8 weeks old) (Table 5). The specific antibody level against both strains remained higher than a 1/45 dilution for up to 28 dpv. Only in two cases, #13549 and #13539 was a long period of immunity not demonstrated, with 1/32 dilution titers observed 28 dpv upon a challenge by serotype Asia 1. However, with respect to the quantification of

Table 4: Evaluation of immune response and protection conferred by epitope-based vaccines VP1Thcomb-FMDV in cattle

Immunize dose[a] (serotype)	No. of cattle	Disease sites	Rate of protections (%)[c]	PD_{50}[d]
Full dose[b] (Asia 1)	13517	-	4/5 (80)	12.51
	13520	Blisters on the tongue		
	13522	-		
	13549	Blisters on the tongue, both forepaw and left hind		
	13539	Blisters on the tongue		
1/3 dose (Asia 1)	13508	-	5/5 (100)	
	13526	Blisters on the tongue		
	13531	-		
	13537	-		
	13086	Lingual ulcers		
1/9 dose (Asia 1)	13509	-	5/5 (100)	
	13512	-		
	13513	-		
	13537	-		
	13524	Lingual ulcers		
Control (Asia 1)	13931	Hooves blister and ulceration	0/2 (0)	
	13109	Hooves and tongue surface blister, ulceration		
Full does[b] (O)	13501	-	5/5 (100)	8.06
	13507	-		
	13312	-		
	13535	Lingual ulcers		
	13518	Lingual ulcers		
1/3 dose (O)	13508	Two foot ulcers	2/5 (40)	
	13526	Lingual ulcers		
	13531	Lingual ulcers, blisters right hind ulcers		
	13537	Lingual ulcers		
	13086	The tongue, lips ulcers, ulcers hooves		
1/9 dose (O)	13509	-	5/5 (100)	
	13512	-		
	13513	-		
	13537	-		
	13524	-		
Control (O)	13931	Ulcers hooves	0/2 (0)	
	13109	Hooves ulcers, tongue ulcers		

[a]All of cattle were challenged with 10,000 BID_{50} of FMDV after vaccination by inoculating the equivalent of a total of 10,000 BID_{50} of FMDV intradermal into two sites on the upper surface of the tongue, [b]Three weeks after vaccination, the vaccinated animals and a control group of 2 non-vaccinated cattle were challenged by inoculating 0.2 mL containing 10,000 BID_{50} (50% bovine infected dose) of homologous virus, [c]Protection was determined that cattle were clinically checked for lesions without any one FMDV-induced lesion in the four feet during observation period (10 days post-challenge). Rate of protection (%) = No. of cattle no lesions/total number of cattle, [d]FMD vaccine potency test PD_{50} (50% protective dose) was calculated by the Reed-Muench method

Table 5: FMDV-specific neutralizing antibody response against Asia 1/O FMDV after inoculation

		Neutralizing antibody[b]								
		-7 dpv[c]		14 dpv		21 dpv		28 dpv		
Challenge[a]	No. of cattle	O	Asia 1	O	Asia 1	O	Asia 1	O	Asia 1	Note[d]
FMDV serotype Asia 1	13517	1/16	1/16	1/256	1/180	1/180	1/180	1/256	1/128	Full dose, Asia 1, protected
	13520	1/16	1/16	1/256	1/180	1/256	1/256	1/256	1/256	Full dose, Asia 1, protected
	13522	1/16	1/16	1/90	1/45	1/180	1/45	1/64	1/32	Full dose, Asia 1, protected
	13549	1/16	1/16	1/32	1/32	1/32	1/32	1/32	1/16	Full dose, Asia 1, non-protected
	13539	1/16	1/16	1/32	1/32	1/45	1/45	1/32	1/32	Full dose, Asia 1, protected
	13512	1/16	1/16	1/256	1/180	1/256	1/180	1/1,024	1/180	1/9 dose, Asia 1, protected
FMDV serotype O	13501	1/16	1/16	1/256	1/90	1/256	1/90	1/256	1/64	Full dose, O, protected
	13507	1/16	1/16	1/256	1/256	1/256	1/256	1/512	1/1,024	Full dose, O, protected
	13535	1/16	1/16	1/256	1/90	1/256	1/90	1/256	1/64	Full dose, O, protected
	13518	1/16	1/16	1/32	1/45	1/90	1/90	1/64	1/128	Full dose, O, protected

[a]Challenged dose in 50 mL unit volume of virus suspension should contain about 100 $TCID_{50}$ (50% tissue culture infective dose) with intramuscular inoculation at the site in the neck, [b]FMDV-specific antibody titer reported as the serum dilution by virus neutralization tests from cattle were vaccinated with peptide vaccine from concentration 100 mg mL^{-1} and challenged 28 days, [c]dpv: Days post vaccination, [d]Animals were challenged at day 8 post-immunization all vaccinated animals were clinically checked for protected effect if they do not develop lesions on the feet. In general, a titer of 1/45 or more of the final serum dilution in the serum/virus mixture is regarded as positive. A titer of less than 1/16 is considered to be negative. For certification of individual animals for the purposes of international trade, titers of 1/16 to 1/32 are considered to be doubtful and further serum samples may be requested for testing

transmission of FMDV among vaccinated cattle, most of the VNT titer against Asia 1 was lower than the titers against O from 7-28 dpv but a single vaccination by VP1Thcomb-FMDV was sufficient to prevent the transmission of serotypes Asia 1 and O of the virus within a group of cattle.

DISCUSSION

Neutralization of the capacity of the FMDV to infect susceptible cells *in vitro* may not be the only functional parameter of the antibody response to an FMDV immunogen that is responsible for protection. However, we tried to compare with conventional vaccine (trivalent inactivated vaccines) data using the same standard potency test protocol[28]. The results in the same period as peptide library VP1Thcomb-FMDV vaccination showed three lots conventional vaccine potency range from 10.81-13.59 PD_{50} a dose for cattle against two stereotypes FMDV including O and Asia 1 (Table 6). When employed for routine prophylactic use, the vaccine should contain at least 3 PD_{50} per dose per cattle by OIE recommended[15,29]. This leads to a conclusion that VP1Thcomb-FMDV peptide library development using immune-informatics tools prediction is feasible for FMD vaccine production[27,30].

Most antigens and vaccines trigger not only B-cell response but also T-cell response. A number of studies have demonstrated potential optimizing in population problem *in silico*[31,32]. However, the best match to build T-epitope design strategies the following optimize the peptides library against FMDV including improved the primary and auxiliary anchor residues to increase the affinity of MHC/peptide complex and extension of peptide length at both ends of epitope core peptides[33]. In the other hand, a web-based tool to predict population coverage of T-cell epitope-based diagnostic and vaccines has been allows retrieving both MHC bind and/or increased affinity for the T cell receptor and cross-reactive T-cells[34]. This peptide preference also provided the general strategy to enhance the affinity MHC-associated immunogenicity epitopes that may introduce alternative, new and effective anchor residues datasets in order to serve as our future optimizing new generation vaccines[35].

The practical implementation for prediction and identification of specific subsets of BoLA-DR3 molecules is to optimize the immune response for populations currently within the species-dependent repertoires of livestock production corporations[4]. Furthermore, peptide-based vaccine can be designed to include alternative antigen determinants from several different pathogens or multiple epitopes of variant stains from the same pathogen[36].

Table 6: Evaluation of protection conferred by conventional vaccines in cattle

Vaccine lot[a]	FMDV serotype	Immune dose (mL)[b]	Rate of protections[c] (%)	PD_{50}[d]
2015008	O	2	5/5 (100)	13.59
		0.67	5/5 (100)	
		0.22	4/5 (80)	
	Asia 1	2	5/5 (100)	10.81
		0.67	5/5 (100)	
		0.22	3/5 (60)	
2015003	O	2	5/5 (100)	11.84
		0.67	4/5 (80)	
		0.22	4/5 (80)	
	Asia 1	2	5/5 (100)	10.81
		0.67	5/5 (100)	
		0.22	3/5 (60)	
2014005	O	2	5/5 (100)	13.59
		0.67	5/5 (100)	
		0.22	4/5 (80)	
	Asia 1	2	5/5 (100)	11.84
		0.67	4/5 (80)	
		0.22	4/5 (80)	

[a]Trivalent inactivated vaccine include OHM/02, AKT-II and Asia KZ/03, [b]Three weeks after vaccination, the vaccinated animals and a control group of 2 non-vaccinated cattle were challenged by inoculating immune dose containing 10,000 BID_{50} (50% bovine infected dose) of homologous virus, [c]Protection was determined that cattle were clinically checked for lesions without any one FMDV-induced lesion in the four feet during observation period (10 days post-challenge). Rate of protection (%) = No. of cattle no lesions/total number of cattle, [d]FMD vaccine potency test PD_{50} (50% protective dose) was calculated by the Reed-Muench method

This study demonstrated that *in silico* approaches to Th epitope selection and design using molecular modeling data concerning MHC with the purpose of rationally designing combinatorial VP1Thcomb-FMDV peptide library to mimic population-spanning Th epitopes can yield Th epitopes that constitutes a promising alternative to traditional vaccine design against FMDV. But thus far, the synthesis of VP1Thcomb-FMDV peptide library offers significant cost savings[37] and stored freeze-dried, which avoids the need to maintain a low temperature situation during storage, transport and distribution[38].

CONCLUSION

Epitope-based vaccines that are chemically well-characterized peptides have become desirable candidate vaccines due to their relative ease of production and construction, chemical stability and their lack of infectious potential. Most traditional vaccines are effective against

viruses that cause acute self-limiting infections, followed by long-lasting immunity. One key aspect of the combinatorial epitope-based vaccines that were designed in this study was the increase in immunogenicity obtained by covering a large fraction of a given target population, which might be optimal for the induction of protective immunity evolved under the variant host immune system. As the availability of combinatorial peptides libraries used for MHC anchors design increases, the elucidation of the structural, molecular and biological basis of the bovine MHC-epitope recognition process and the rational design of combinatorial epitope-based vaccines may see an increase in sophistication, potentially resulting in more cost-efficient development of peptide vaccine campaigns that minimize the need for FMD control by culling. More generally, designed epitope-based vaccines may offer a potential route for the development of vaccines that target not only FMDV but many other pathogens in cattle.

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REFERENCES

1. Mulcahy, G., C. Gale, P. Robertson, S. Iyisan, R.D. DiMarchi and T.R. Doel, 1990. Isotype responses of infected, virus-vaccinated and peptide-vaccinated cattle to foot-and-mouth disease virus. Vaccine, 8: 249-256.
2. Thiermann, A.B., 2015. International standards: The world organisation for animal health terrestrial animal health code. Rev. Sci. Technol., 34: 277-281.
3. Patronov, A. and I. Doytchinova, 2013. T-cell epitope vaccine design by immunoinformatics. Open Biol., Vol. 3. 10.1098/rsob.120139
4. Schubert, B., O. Lund and M. Nielsen, 2013. Evaluation of peptide selection approaches for epitope-based vaccine design. Tissue Antigens, 82: 243-251.
5. Wang, C.Y., T.Y. Chang, A.M. Walfield, J. Ye and M. Shen *et al*., 2002. Effective synthetic peptide vaccine for foot-and-mouth disease in swine. Vaccine, 20: 2603-2610.
6. Eisenberg, D., R.M. Weiss and T.C. Terwilliger, 1982. The helical hydrophobic moment: A measure of the amphiphilicity of a helix. Nature, 299: 371-374.
7. Lafuente, E.M. and P.A. Reche, 2009. Prediction of MHC-peptide binding: A systematic and comprehensive overview. Curr. Pharmaceut. Des., 15: 3209-3220.
8. Zhang, Q., P. Wang, Y. Kim, P. Haste-Andersen and J. Beaver *et al*., 2008. Immune epitope database analysis resource (IEDB-AR). Nucl. Acids Res., 36: W513-W518.
9. Sturniolo, T., E. Bono, J. Ding, L. Raddrizzani and O. Tuereci *et al*., 1999. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. Nat. Biotechnol., 17: 555-561.
10. Houghton, C.S.B., M.E. Engelhorn, C. Liu, D. Song and P. Gregor *et al*., 2007. Immunological validation of the EpitOptimizer program for streamlined design of heteroclitic epitopes. Vaccine, 25: 5330-5342.
11. Garcia-Briones, M.M., G.C. Russell, R.A. Oliver, C. Tami and O. Taboga *et al*., 2000. Association of bovine *DRB3* alleles with immune response to FMDV peptides and protection against viral challenge. Vaccine, 19: 1167-1171.
12. Vita, R., J.A. Overton, J.A. Greenbaum, J. Ponomarenko and J.D. Clark *et al*., 2015. The immune epitope database (IEDB) 3.0. Nucleic Acids Res., 43: D405-D412.
13. Rice, P., I. Longden and A. Bleasby, 2000. Emboss: The european molecular biology open software suite. Trends Genet., 16: 276-277.
14. Alam, M.J. and K.U.M. Ashraf, 2013. Prediction of an epitope-based computational vaccine strategy for gaining concurrent immunization against the venom proteins of australian box jellyfish. Toxicol. Int., 20: 235-253.
15. Fry, E.E., J.W.I. Newman, S. Curry, S. Najjam and T. Jackson *et al*., 2005. Structure of Foot-and-mouth disease virus serotype A1061 alone and complexed with oligosaccharide receptor: Receptor conservation in the face of antigenic variation. J. Gen. Virol., 86: 1909-1920.
16. Bolin, D.R., A.L. Swain, R. Sarabu, S.J. Berthel and P. Gillespie *et al*., 2000. Peptide and peptide mimetic inhibitors of antigen presentation by HLA-DR class II MHC molecules. Design, structure-activity relationships and X-ray crystal structures. J. Med. Chem., 43: 2135-2148.
17. Sali, A. and T.L. Blundell, 1993. Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol., 234: 779-815.
18. Morris, G.M., R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell and A.J. Olson, 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem., 30: 2785-2791.
19. Biasini, M., S. Bienert, A. Waterhouse, K. Arnold and G. Studer *et al*., 2014. SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. Nucl. Acids Res., 42: W252-W258.
20. Ellis, S.A., R.E. Bontrop, D.F. Antczak, K. Ballingall and C.J. Davies *et al*., 2006. ISAG/IUIS-VIC comparative MHC nomenclature committee report, 2005. Immunogenetics, 57: 953-958.
21. Weisel, M., E. Proschak and G. Schneider, 2007. PocketPicker: Analysis of ligand binding-sites with shape descriptors. Chem. Cent. J., Vol. 1. 10.1186/1752-153X-1-7

22. Pizzi, M., 1950. Sampling variation of the fifty per cent end-point, determined by the reedmuench (behrens) method. Hum. Biol., 22: 151-190.
23. Cooke, J.N. and K.M. Westover, 2008. Serotype-specific differences in antigenic regions of foot-and-mouth disease virus (FMDV): A comprehensive statistical analysis. Infect Genet. Evol., 8: 855-863.
24. Wang, P., J. Sidney, Y. Kim, A. Sette, O. Lund, M. Nielsen and B. Peters, 2010. Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinform., Vol. 11. 10.1186/1471-2105-11-568
25. Schafroth, H.D. and C.A. Floudas, 2004. Predicting peptide binding to MHC pockets via molecular modeling, implicit solvation and global optimization. Proteins, 54: 534-556.
26. Dessen, A., C.M. Lawrence, S. Cupo, D.M. Zaller and D.C. Wiley, 1997. X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II. Immunity, 7: 473-481.
27. Hammer, J., C. Belunis, D. Bolin, J. Papadopoulos and R. Walsky *et al*., 1994. High-affinity binding of short peptides to major histocompatibility complex class II molecules by anchor combinations. Proc. Natl. Acad. Sci. USA., 91: 4456-4460.
28. Hassan, A.I., 2016. Effect of different culture systems on the production of foot and mouth disease trivalent vaccine. Vet. World, 9: 32-37.
29. Grubman, M.J. and B. Baxt, 2004. Foot-and-mouth disease. Clin. Microbiol. Rev., 17: 465-493.
30. Bui, H.H., J. Sidney, W. Li, N. Fusseder and A. Sette, 2007. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinform., Vol. 8. 10.1186/1471-2105-8-361
31. Sakib, M.S., M.R. Islam, A.K.M.M. Hasan and A.H.M. Nabi, 2014. Prediction of epitope-based peptides for the utility of vaccine development from fusion and glycoprotein of nipah virus using *in silico* approach. Adv. Bioinform. 10.1155/2014/402492
32. Soria-Guerra, R.E., R. Nieto-Gomez, D.O. Govea-Alonso and S. Rosales-Mendoz, 2015. An overview of bioinformatics tools for epitope prediction: Implications on vaccine development. J. Biomed. Inform., 53: 405-414.
33. Godkin, A.J., K.J. Smith, A. Willis, M.V. Tejada-Simon, J. Zhang, T. Elliott and A.V. Hill, 2001. Naturally processed HLA class II peptides reveal highly conserved immunogenic flanking region sequence preferences that reflect antigen processing rather than peptide-MHC interactions. J. Immunol., 166: 6720-6727.
34. Peters, B., J. Sidney, P. Bourne, H.H. Bui and S. Buus *et al*., 2005. The immune epitope database and analysis resource: From vision to blueprint. PLoS Biol., Vol. 3. 10.1371/journal.pbio. 0030091
35. Berzofsky, J.A., J.D. Ahlers and I.M. Belyakov, 2001. Strategies for designing and optimizing new generation vaccines. Nature Rev. Immunol., 1: 209-219.
36. Purcell, A.W., J. McCluskey and J. Rossjohn, 2007. More than one reason to rethink the use of peptides in vaccine design. Nature Rev. Drug Discov., 6: 404-414.
37. Correia, B.E., J.T. Bates, R.J. Loomis, G. Baneyx and C. Carrico *et al*., 2014. Proof of principle for epitope-focused vaccine design. Nature, 507: 201-206.
38. Lloyd, J., S. McCarney, R. Ouhichi, P. Lydon and M. Zaffran, 2015. Optimizing energy for a green vaccine supply chain. Vaccine, 33: 908-913.