Serological Detection of FMD Serotypes by New Prepared Innovative Recombinant Hepta-Epitopic Peptide

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Abstract:

BACKGROUND: Foot-and-mouth disease (FMD) is a highly contagious and economically important disease that affects cloven-hoofed animals worldwide. In recent years, a series of outbreaks of FMD have occurred in many countries. Recombinant protein synthesis incorporating protective B- and T-cell epitopes are candidates for new safer and more effective (FMD) vaccines that have potential to provide protective immunity against diverse FMDV strains and to protect against future epidemics.

OBJECTIVES: The aim of this study was to produce Recombinant Hepta-epitope peptide for detecting FMD O, A, Asia1 serotypes, which can be used as a tool for diagnostic kits.

METHODS: In the present study, we designed and produced a Recombinant Hepta-epitopic peptide from FMDV epitopes of the viral proteins VP1, VP2, VP3, 3C (seven epitopic regions, amino acid residues VP1 140-160, Vp1 200-213, VP2 68-75, VP2 179-198, VP3129-148, VP3 190-199, 3C121-135).

RESULTS: It was shown that the mentioned recombinant peptide could recognize the serum collected from cattle infected with FMD serotype A and O.

CONCLUSIONS: We believe that this recombinant Hepta-epitopic peptide can be used for diagnostic serological assays. Furthermore, this Recombinant peptide may be a potential candidate as an alternative vaccine against FMDV epidemic variants.

Keywords:

Diagnostic serological assays, Epitope, FMD, Recombinant hepta-epitope peptide, Synthetic peptides vaccine

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Introduction

Foot-and-mouth disease virus (FMDV) belongs to the genus Aphthovirus within the family Picornaviridae that causes a highly contagious disease of cloven-hoofed farm animals (Pereira 1981). Although many researchers have tried to control FMD, FMD virus (FMDV) is difficult to eradicate because of its rapid mutation and variation. Seven different serotypes of FMDV (O, A, C, Asia-1, SAT-1, SAT-2, and SAT-3) have been identified (Domingo, Baranowski et al. 2002, Lee, Park et al. 2015) . Serotype O is known as the main serotype of FMDV that has broken out in East Asia, Middle Asia, Africa and Europe (Samuel and Knowles 2001).

The FMDV particles contain a positive strand RNA molecule of about 8,500 nucleotides, enclosed within an icosahedral capsid comprising 60 copies each of four virus proteins VP1 to VP4 (Bachrach 1977). The genome encodes a unique polyprotein in which different viral polypeptides are cleaved by viral proteases(3C) (Sanz-Parra, Jimenez-Clavero et al. 1999), including nine different mature structural and nonstructural proteins (NSP). Each of these NSP, as well as some of the precursor polypeptides are involved in functions that are relevant to the virus life cycle in infected cells.

The basic control of Foot and Mouth disease is dependent on preventive policies and extensive vaccination of all susceptible individuals (Brown 1992).

Conventional FMDV vaccines are formulated with inactivated virus. For the production of such vaccines enormous amounts of the infectious agent are needed and therefore represent a serious risk of viral dispersion (Brown 1992, Brown 1992).

The recombinant immunogenic poly peptides with selected epitopes as an alternative vaccine would prevent the risk of infection in the vaccinated animals. Moreover, Recombinant protein synthesis vaccines do not present safety risks such as reversion or persistence of incomplete inactivation (Brown 1992), or even integration into the host cell genome as in the case of naked DNA vaccines (Klinman, Takeno et al. 1998).

It must be mentioned that especially in Foot and Mouth disease, Recombinant protein synthesis vaccines have also been able to confer a broader heterotypic protection than those formulated with the whole inactivated virus (Doel, Gale et al., 1990). Amino acid residues are recognized by T-lymphocytes and B-lymphocytes in order to induce an effective immunity (Berzofsky 1995, Kabeya, Ohashi et al. 1996). Although Four structural proteins (VP1, VP2, VP3 and VP4) comprise the viral capsid of FMDV, many studies show that the main immunogenic protein was VP1(Collen, Dimarchi et al. 1991, Garcia-Valcarcel, Doel et al. 1996, Volpina, Surovoy et al. 1999). On the other hand, the results obtained in cattle upon vaccination with Recombinant protein synthesis containing only VP1 epitopes were in many cases insufficient in terms of the immune response (Doel, Gale et al. 1990, Glass and Millar 1995) or the protection achieved in vaccination trials (Doel, Gale et al. 1990). A continuous, immunodominant B-cell site located in the GH loop, around positions 140 to 160 of capsid proteinVP1, has been widely used as an immunogenic peptide (Bittle, Houghten et al. 1982). Some studies presented the heterotypic lymphoproliferative responses against different NSP,

using lymphocytes from FMDV infected pigs. For the NSP 3A, 3B, and 3C, which consistently induced higher responses, overlapping Recombinant protein synthesis was employed to identify MHC class II-restricted T-cell sites.(Blanco, Garcia-Briones et al., 2001)

In the present study, we designed and produced a Recombinant Hepta-epitopic FMDV Peptide composed of the viral proteins VP1, VP2, VP3, 3C (seven epitopic regions VP1-140-160, Vp1-200-213,VP2 68-75,VP2 179-198,VP3129-148,VP3 190-199, 3C121-135). Our results in this study will provide a recombinant Hepta-epitopic peptide that can be used for diagnostic serological assays. Also, this immunogenic recombinant antigen can be considered as a candidate for the creation of a recombinant vaccine.

Material and Methods

Bioinformatic Design of Recombinant Hepta-epitope peptide: Sixty peptide sequences of FMD viruses O serotype isolated from Iran,Turkey, Afghanistan, Pakistan were collected from NCBI database, and were aligned for analyzing the constant region in the different sequences. Based on cell epitope prediction servers such as Protparam, IEDB, Bepipred, Discotope, Ellipro, Epitopia and MHC-Pred, the predicted B-cell and T-cell epitopes were performed. Within recognized epitopes, seven epitopic regions (three B-cell epitopes and four T-cell epitopes) of type O FMDV were selected (amino acid residues 140-160 and 200-213 from VP1), (amino acid residues 68-75 and 179-198 from VP2), (amino acid residues 190-199 and 129-148 from VP3) and 121-135 from 3C (Fig. 1). A 970 base pair (bp) synthetic nucleotide sequence coding for the above mentioned epitopes was designed and produced by (MWG Company, Germany).

Preparation of Recombinant Hepta-epitope peptide: This 970 base pair (bp) sequence was synthesized and cloned in pex-A2 plasmid by MWG Company, Germany. For elevating efficacy each epitope was designed with three or four tandem repeats. To minimize interference between adjacent epitopes, each epitope was separated by five glycine. Additionally, nucleotide sequences for 6 Histidine was added to the end of the sequence for separating easily. The cloning result was confirmed by PCR analysis. Subsequently, the recombinant plasmid was transferred into E. coli BL21 (DE3) (Novagen, CA, USA) and recombinant colonies were selected with isopropyl-β-D-thiogalactopyranoside (IPTG) and X-gal. White colonies were isolated and confirmed by PCR method.

PCR-Analysis: To confirm the recombinant plasmid, the cloned DNA segment was amplified with the specific primers F:5′-TCGCTTGTACCAATGCTTACAG-3′ and R:5′-GGTAAGCTTTTATTATGTGAG-3′. The PCR reaction was carried out in a final volume of 25 µl, 12.5 µl of Amplicon Master Mix, 1 µl of each primer (10 µM) and 50 ng of the cloned plasmid. PCR reaction was done in a Thermocycler device (Primus, Germany), according to the PCR program, denaturation at 95 °C for 3 min, 30 cycles were programmed as follows: 94 °C for 45 s, 58 °C for 45 s, 72 °C for 60s, and the final expansion step was carried out at 72 °C for 5 min. The PCR product was loaded onto a 1% agarose gel.

Recombinant Hepta-epitope peptide Expression: The recombinant colonies cul-

tured in LB broth and protein expression were induced by 1 mM IPTG. The amount of produced protein was measured after 4 h and 6 h after induction. Then the Recombinant peptide was extracted from bacterial cell pellet by sonication on ice (48 × 10 s) and freezing–thaw three times for 5 min in liquid nitrogen and boiling water. Lysates were centrifuged at 17,000 rpm at 4 °C for 20 min. After that, Recombinant Hepta-epitope peptide in supernatants was purified using Ni-nitrilotriacetic acid (NTA) agarose resin (Qiagen, Germany) as described by company instructions.

The recombinant Hepta-epitopic peptide was analyzed once by Dot blotting using anti-His-tag antibody (1:1000, CytoMittogene, Iran) in peroxidase system. Subsequently, the expressed protein pattern was analyzed by15 % SDS-PAGE and the recombinant peptide was confirmed by western blot assay using anti-FMDV IgG antibody. Briefly, the lysate proteins were separated on a 15% SDS–PAGE and then transferred to a nitrocellulose membrane (Whatman, Germany). The membrane was blocked by 1% BSA in Phosphate buffered saline (PBS) contacting 0.05% Tween 20 (PBST) for 1 h on a shaker and then washed three times with PBST. The membrane was incubated with a 1:1000 diluted anti-FMDV IgG antibody overnight at 4 °C, washed three times with PBST, and incubated with a 1:2000 dilution of anti antibody conjugated with horseradish peroxidase (HRP) for1 h. After washing three times with PBST, the signal was developed and stained using 3'-Diaminobenzidine (DAB) substrate.

Analysis of Recombinant Hepta-epitope peptide by ELISA assay: The isolated recombinant protein was analyzed by antigen sandwich ELISA kit ([IZSLER], Brescia, Italy, an OIE/FAO FMD reference laboratory) which can detect pan-FMDV and serotypes O, A(subtype 4D12and 5F6), Asia1, and C according to the manufacturer's instructions. Briefly, micro plates were supplied pre-coated with catching specific type O, A, Asia1, C MAbs with a universal pan-FMD MAb and also with positive controls already incorporated onto plates. 25µl of six samples, the recombinant protein in two dilutions named Pro1 and Pro2 (concentration of purified recombinant antigen was 972µg/ml that was diluted to 2/3, 1/2) and four tongue epithelium suspension samples of affected cows (A4, A5, A42, A43), were incubated with the coated MAbs. The FMD virus present in samples or recombinant Hepta –poly peptide were captured by the type-specific MAb and by an universal pan-FMDV MAb. After washing to remove unbound materials, a unique pan-FMD MAbs, peroxidase-conjugated, was added (as a detector). After incubation, the unbound conjugate was removed by washing. The reaction was assessed using TMB-chromogenic solution. The colorimetric reaction develops if the conjugate has bound to the sample antigen. The color development is proportional to the amount of viral antigen present in the test sample. After addition of the stop solution, the optical density of the developed color was read by a micro plate ELISA-reader.

Collection and detection of FMD positive cattle sera by ELISA: Forty-six sera were collected from 40 FMD suspected cattle and 6 control cattle in Khorasan province (samples A1- A46) and analyzed with FMDV 3ABC - TRAPPING ELISA kit ([IZSLER], Brescia, Italy). This kit is a trapping-indirect ELISA for the detection of antibodies to the non-structural polypep-

tide 3ABC of FMD virus in serum samples of large and small ruminants. This kit uses an anti-3A specific monoclonal antibody (MAb) coated to the solid phase to trap the recombinant 3ABC polypeptide expressed in E. coli. Briefly, the micro titer plates were supplied pre-coated with the3ABC antigen captured by the affected animal sera. Appropriately diluted test sera were incubated with the trapped antigen, enabling the specific antibodies eventually present in the sample to bind to the 3ABC. After washing to remove unbound material, the anti-ruminant IgG, peroxidase-conjugated Mab was added into the micro titer plates. After incubation, the unbound conjugate is removed by washing, and the TMB-chromogen substrate was delivered into the wells. A colorimetric reaction was estimated by the optical density (OD) at 450 nm.

Percentage positivity= net OD value of test serum & controls / net OD value of positive control serum(PCS) ×100

Evaluation of Recombinant Hepta-epitope peptide: An Indirect Sandwich Assay was developed for evaluation of the recombinant polypeptide. For this aim, Pirbright FMDV type O, A, Asia 1 ELISA kit with some changes (OIE/FAO FMD reference laboratory, England) was used(in this kit rabbit antisera specific for FMDV serotypes O, A, Asia1 was supplied for trapping antigen, instead of rabbit antisera, cattle's positive sera that were selected in the test above were coated for trapping Ag, so that in each plate 4 positive sera samples were coated and each cow serum sample was coated in 2 rows. Briefly, 96 well micro titer plate was coated with 50 µmole/well positive serum samples that were selected in the test above (instead of MAb in the manufacturer's protocol)(diluted 2:1000 in PBST/0.05% tween 20) in carbonate-bicarbonate buffer (CBB) for 1 h at 37 °C. Subsequently, the unbound areas of the bottom of the micro titer plate were blocked with 0.5% skim milk in PBS for 1 h at 37 °C. After that, the wells were washed three times with PBS. The positive control antigen O, A was added into the wells 1 to 4 as positive control and the wells 5 to 6 were used as negative control. Then, 50µl recombinant polypeptide samples were added in the wells 7 to 12 (concentration of purified recombinant antigen was 972µg/ml). The end volume of the solution in the wells of the micro titer plate was adjusted to 100 µl with PBST (0.05% tween 20 in PBS) containing 0.5% skim milk. Plates were incubated for 1 h at 37 °C. After washing the wells, the detecting antibody (MAb serotype O, A diluted 1:100) was added into the wells and incubated for 1 h at 37 °C (horizontally MAb for each serotype O or A was added to each row of each sample).Then the wells were washed three times. Next, HRP conjugated anti antibody diluted 1: 200 in PBST containing 0.5% skim milk was added into the wells and incubated for 2h at room temperature. The reaction was visualized using OPD chromogen (100 µl/well) and stopped by an equal volume of 0.18 M H2SO4. The reaction was estimated at 492 nm in a Micro spectrophotometer (Biotech, USA).

Results

Preparation of Recombinant Hepta-epitope peptide: We gathered sequence information of VP1,VP2, VP3 and 3C of FMDV type O, at NCBI GenBank and analyzed the amino acid sequence similarity of various peptides. Then, based on B and T Cell epitope prediction seven epitopic regions (amino acid residues 140-160 and

Table 1. The used regions used for the preparing the poly epitopic peptide (B cell epitopes and T cell epitope).

NO	region	Amino acid region	Epitope prediction
E1	VP1	140-160	B,T cell epitope
E2	VP1	200-213	T cell epitope
E3	VP2	68-75	T cell epitope
E4	VP2	179-198	B,T cell epitope
E5	VP3	129-148	B,T cell epitope
E6	VP3	190-199	B,T cell epitope
E7	3C	121-135	T cell epitope

200-213 from VP1), (amino acid residues 68-75 and 179-198 from VP2), (amino acid residues 190-199 and 129-148 from VP3) and 121-135 from 3C were selected (Table 1). The nucleotide sequence of recombinant Hepta-epitope peptide was designed with the seven selected peptides (Table 1) and cloned into pEX-A2 plasmid. Then the cloned plasmid was transformed to E. coli BL21 and cultured on Amp LB medium with IPTG –Xgal. White colonies were selected and confirmed using PCR method. The image of gel electrophoresis of the cloned gene showed a band of about 1000 bp (Fig. 1).

The confirmed colonies were cultured in LB broth for 4h and some of them for 6h. After that, expressed protein was extracted and his-tag purification by Ni-NTA agarose kit was performed.

The concentration of the acquired protein was measured by Bradford method. It was determined that purified Hepta-epitopic peptide was produced by 972 µg per milliliter culture.

Characterization of Recombinant Hepta-epitope peptide: Recombinant Hepta-epitope peptide, was analyzed by Dot blotting using anti-His-tag antibody (Fig. 2), and result showed that the Recombinant peptide is located in the cell suspension.

Then size of polypeptide was shown by SDS-PAGE method. In this method the expression pattern of recombinant proteins in BL21 culture was observed (Fig.3 a) M, PageRuler™ Prestained Protein Ladder, 10 to 180 kDa ThermoFisher, and Hepta-epitopic Protein was detected about 33000 Dalton. Recombinant peptide analysis was followed by western blotting using anti-FMDV monoclonal antibody (Fig.3b). That shows the Rec-protein can be recognized by anti-FMDV antibody. Also, the result indicated that the amount of produced protein after 4 h is more than 6 h after inoculation.

Antigenic evaluation of Recombinant Hepta-epitope peptide: Sera from 40 cattle suspected of FMD and 6 control cattle were analyzed with FMDV 3ABC-Trapping ELISA kit. Sera from all 40 cattle suspected of FMD were as expected positive and all 6 control cattle were negative (Table 3). Table 3 showed different FMD antibody titer by examined sera. For further examination, four samples were prepared from tongue epithelium of two FMD positive cattle (A4 and A5) and two FMD negative cattle (A42 and A43). These four samples and the recombinant Hepta-epitopic peptide in two concentrations were analyzed with serotype specific antibodies [Type O, Type A (MAb4D12), Type A (5F6), Type Asia 1, Type C and Pan-FMD] by ELISA. The results showed that the recombinant poly epitopic peptide could be recognized with antibodies against serotypes Type O, Type A (MAb4D12), Type A (5F6), Type Asia 1 and Pan-FMD but not with Type C in both concentrations. The samples A4 and A5 could be recognized only with anti Type O and anti Type A (MAb4D12) and Pan-FMD but not with other examined anti serotypes. As expected, the samples prepared from con-

Table 2. The recombinant Hepta-epitoic peptide in two concentrations and 2 samples of FMD infected cattle (A4 and A5) and 2 samples prepared from tongue epithelium of control cattle (A42 and A43) were analyzed with serotype specific antibodies [Type O, Type A (MAb4D12), Type A (5F6), Type Asia 1, Type C and Pan-FM] by ELISA.

	Monoclonal Ab serotype detection					
samples	Type O	Type A MAb4D12	Type A 5F6	Type Asia 1	Type C	Pan-FMD
Pro1	Pos	pos	pos	pos	neg	Pos
pro 2	pos	pos	pos	Pos	neg	Pos
A4	pos	pos	neg	neg	neg	Pos
A5	pos	pos	neg	neg	neg	Pos
A42	neg	neg	neg	neg	neg	neg
A43	neg	neg	neg	neg	neg	neg
Positive control	Pos	Pos	Pos	Pos	Pos	Pos
Negative control	neg	neg	neg	neg	neg	neg

trol cattle were negative with all examined antibodies (Table 2).

In the next experiment, 40 positive sera that are mentioned above were used for trapped hepta-epitopic peptide as an Ag in an Indirect Sandwich Enzyme-Linked Immunosorbent Assay, it could be shown that all 40 positive sera could recognize the Recombinant Hepta-epitope peptide.

Discussion

We chose seven epitopes from four antigenic regions VP1, VP2, VP3 and 3C. One of the most important antigenic regions is VP1 GH loop that when presented with VP2, VP3 and 3C as a tandem repeat can induce CD4+ T helper-cell and also CD8+ lymphocytes in the protection against FMD infection. Dendrimeric peptides including four copies of a B-cell epitope (VP1, 136 to154) linked to a T-cell epitope (3A, 21to35) of FMD virus (FMDV) elicit potent B-and T-cell specific responses and confer protection to viral challenge, while juxtaposition of these epitopes in a linear peptide induces less efficient responses. They showed the bivalent (B2T) 2 B cell and 1 T cell construction elicited similar or even better B-and T-cell specific responses than tetravalent B 4T. In addition, the presence of the T-cell epitope and its orientation were shown to be critical for the immunogenicity of the linear juxtaposed monovalent peptides analyzed in parallel (Blanco, Andreu et al., 2017).

To further verify the Recombinant Hepta-epitope peptide, it was generated using a reverse genetic system according to bioinformatics result of sequence analysis of VP1and full genome of FMDV virus Type O isolated from Iran,Turkey, Afghanistan, Pakistan that the corresponding nucleotide sequences were still registered in NCBI database.

Our results showed that, the Recombinant Hepta-epitope peptide could be recognized by all antisera against A, O, Asia 1.Wang et al. (2012), indicated that the epitope-inserted virus has the potential to induce neutralizing antibodies against both FMDV type Asia1 and type O, and their results demonstrated that the G-H loop of FMDV type Asia1 effectively display the protective neutralizing epitopes of other FMDV serotypes, making this an attractive approach for the design of novel FMDV vaccines (Wang, Xue et al. 2012).

In the present study we conducted an experiment to test the efficacy of the recom-

Table 3. Sera from 40 cattle suspect to FMD and 6 control cattle were analyzed with FMDV 3ABC-Trapping ELISA kit.

Sample ID	% Positivity	Result
A1	52.553	Positive
A2	71.171	Positive
A3	30.631	Positive
A4	174.399	Positive
A5	154.054	Positive
A6	168.994	Positive
A7	123.273	Positive
A8	164.79	Positive
A9	183.559	Positive
A10	187.462	Positive
A11	178.003	Positive
A12	167.117	Positive
A13	120.12	Positive
A14	146.997	Positive
A15	180.556	Positive
A16	155.03	Positive
A17	137.462	Positive
A18	167.267	Positive
A19	122.673	Positive
A20	125.375	Positive
A21	92.117	Positive
A22	150.15	Positive
A23	137.688	Positive
A24	125.601	Positive
A25	177.928	Positive
A26	160.06	Positive
A27	137.613	Positive
A28	178.228	Positive
A29	112.838	Positive
A30	89.79	Positive
A31	150.375	Positive
A32	157.583	Positive
A33	137.688	Positive
A34	125.601	Positive
A35	157.583	Positive
A36	137.613	Positive
A37	177.228	Positive
A38	113.838	Positive
A39	124.673	Positive
A40	125.775	Positive
A41	9.384	Negative
A42	1.727	Negative
A43	1.802	Negative
A44	7.883	Negative
A45	4.279	Negative
A46	5.03	Negative

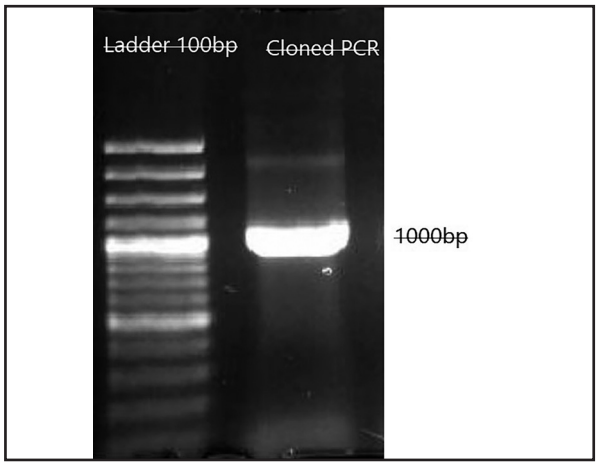

Figure 1. The recombinant plasmid was amplified by PCR to show PCR product of cloned gene.

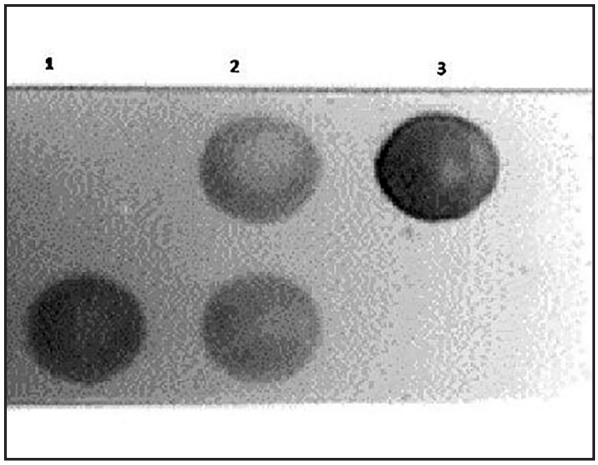

Figure 2. Recombinant Hepta-epitopic Peptide (Rec peptide), was analyzed by Dot blotting using anti-His-tag antibody (upper line: 1- culture supernatant, 2-cell supernatant , 3-Concentrated purified Rec peptide, lower line : 1- purified Rec peptide,2- cell supernatant, 3-cell debris).

binant protein by ELISA according to the FMD positive sera of affected cattle. Our results showed that the recombinant poly epitopic peptide could be recognized with antibodies against serotypes Type O, Type A (MAb4D12), Type A (5F6), Type Asia 1 and Pan-FM but not with Type C. Also as expected, sera collected from 40 cattle infected with FMD virus could recognize the recombinant Hepta epitopic peptide, which denoted that the recombinant peptide had wide spectrum for heterologous FMD virus. Antigen-capture sandwich ELISA has

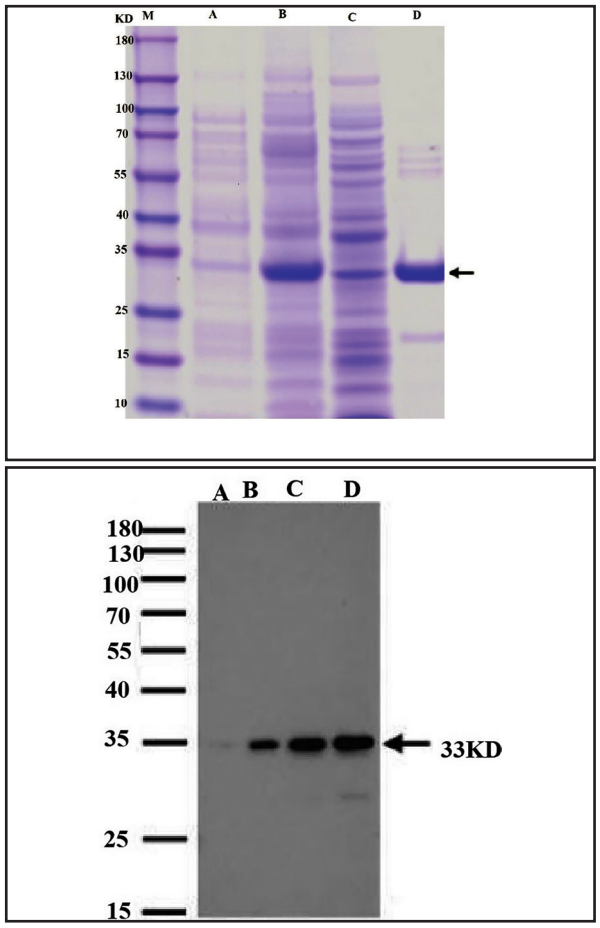

Figure 3. A, 15% SDS-page gel stained with Coomassie Brilliant Blue, shows the Recombinant protein extracted and purified - M protein marker PageRuler™ Prestained Protein Ladder, 10 to 180 kDa. A-culture suspension, B-cell suspension (4houres), C-cell suspension(6houres), D- purified Rec peptide, (Protein size is about 33 kDa). B, Western blot analysis recombinant proteins were detected with antiFMDV antibody. A-cell suspension, B- cell suspension (6houres), C-cell suspension(4houres) D-purified Rec protein.

100% specificity for heterologous FMDV and 80% sensitivity for detection of complete virus particles in clinical samples (Giridgharan et al., 2005).

By reviewing other articles, we will get similar results , for example, Ko et al produced a recombinant protein (rP13C) that was derived from the P1 precursor and 3C protease genes that were cloned into a single expression vector and expressed in insect cells. This protein elicited a low titer of FMDV neutralizing antibodies in pigs. They demonstrated that an ELISA using recombinant proteins has the potential to replace the liquid phase blocking (LPB) ELISA using an inactivated FMDV antigen as a simple and robust serological tool for screening antibodies to FMDV serotype Asia 1 (Ko, Jeoung et al. 2009). Furthermore, expression of structural polypeptide of FMDV (serotype A) using a baculovirus and assembled into empty virus-like particles (VLPs) showed antigenicity comparable to chemically inactivated FMDV (Basagoudanavar, Hosamani et al. 2013). Biswal et al. (2015) developed a recombinant capsid polyprotein (rP1) of FMD virus (FMDV) serotype O and used it as an antigen for the detection of antibodies to FMDV. They used the rP1protein for protein-based solid phase competitive ELISA (rP1-SPCE) and compared the performance of the rP1-SPCE with in-house, liquid-phase blocking ELISA (LPBE). So they suggested this recombinant capsid polyprotein-based ELISA for detection of antibodies to FMDV serotype O(Biswal, Bisht et al. 2015).

Also, expression of 3ABC poly-protein in Escherichia coli system for optimization of two formats of enzyme immunoassays (sandwich and competitive ELISAs) to differentiate FMD infection among the vaccinated population, showed determination power of Diagnostic sensitivity/ specificity of sandwich and competitive ELISAs by ROC method is 92.2%/95.5% and 89.5%/93.5% (Sharma, Mahajan et al. 2014).

In this study, we produced recombinant DNA and Hepta-epitopic peptid,which contain both the B cell and T cell epitopes. The nucleotide sequences were codon-optimized, and its expression was confirmed in both mammalian cells and bacterial system.

The purified peptides were found to have antigenicity power and to bind to cattle anti FMD antibodies so it could be presented as a recombinant vaccine after more experiments. Many articles highlighted the necessity of producing FMD peptide vaccines and their high ability of protection. Many efforts are currently devoted to developing novel vaccines including recombinant protein vaccines, synthetic peptide vaccines, and empty capsid vaccines (Cao, Lu et al. 2016) (Lyons, Lyoo et al. 2016).

Also, incorporation of a FMDV specific T-cell epitope in the peptide formulation allows a significant reduction in virus excretion and clinical score after challenge (Cubillos, de la Torre et al. 2012). Dong et al. (2015) expressed the coding genes of 141-160 epitope peptide of VP1 in the coat protein (CP) genes of MS2 in prokaryotic expression vector, and the recombinant protein self-assembled into virus-like particles (VLP) and showed that the CP-EP141-160 VLP had a strong immunoreaction with the FMD virus (FMDV) antigen in vitro, and also had an effective immune response in mice (Dong, Zhang et al. 2015).

Given the ability of the Recombinant Hepta-epitope peptide to identify specific antibodies, it can be concluded that the antigenicity has an appropriate antigenicity and hence, if used as a vaccine, can stimulate the existing immunity, especially for cattle. Also, we found that 40 of the positive cases responded to the anti-serotype antibodies indicating the proper function and ability of the recombinant protein in response to each of the three circulating strains A, O, Asia1, and could show the strength of immunization against the three serotypes.

In conclusion, the results showed that the Recombinant Hepta-epitopic peptide could be recognized with all three common serotypes A, O and Asia1. This means that this antigen has strong and effective antigenicity which can make it a suitable candidate for vaccine development against FMD.

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بررسی سرولوژیکی سروتایپ‌های تب برفکی با استفاده از پپتید نوترکیب هفت اپی توپی نوآوری شده

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چکیده

زمینه مطالعه: بیماری تب برفکی (FMD) یک بیماری بسیار مسری وبا اهمیت از نظر اقتصادی است که حیوانات فراوانی رادر سراسر جهان مبتلا کرده است. در سال‌های اخیر، مواردی ازشیوع FMD در بسیاری از کشورها رخ داده است. پپتیدهای نوترکیب حاوی اپی توپ‌های محافظتی B- cell و T-cell کاندیدی برای واکسن‌های جدید ایمن تر و موثرتر (FMD) هستند که توانایی ایجاد ایمنی محافظتی را در برابر انواع سروتایپ‌های FMDV داشته و قادرند ما را در برابر اپیدمی‌های آینده محافظت کنند.

هدف: هدف ما از این تحقیق تولید یک پپتید هفت اپی توپی برای شناسایی سروتایپ‌های A, O, Asia۱ بیماری تب برفکی است که بتواند بعنوان ابزاری جهت کیت‌های تشخیصی بکار رود.

روش کار: در این مطالعه یک هپتا پپتید نوترکیب حاوی اپی توپ‌های نواحی VP۱, VP۲, VP۳, ۳C طراحی وتولید گردید .این مناطق شامل هفت ناحیه اپی توپی (VP۱-۱۴۰-۱۶۰ ، Vp۱-۲۰۰-۲۱۳ ، VP۲ ۶۸-۷۵ ، VP۲ ۱۷۹ -۱۹۸ ، VP۳۱۲۹-۱۴۸، VP۳ ۱۹۰-۱۹۹ ، ۳C۱۲۱-۱۳۵) می‌باشد.

نتایج: آزمایشات سرولوژی انجام شده به روش الایزا در این تحقیق نشان داده است که پپتید نوترکیب مورد نظر می‌تواند سرم‌های جمع آوری شده از گاوهای مبتلا به سروتایپ‌های A و O تب برفکی را شناسایی کند.

نتیجه‌گیری نهایی: بنابراین ما معتقدیم که این هپتا پپتید نوترکیب می‌تواند به عنوان ابزاری برای آزمایش‌های تشخیصی سرولوژیکی بیماری تب برفکی استفاده شود. علاوه بر این، پروتئین نوترکیب چند اپی توپی طراحی شده در این مطالعه ممکن است نامزدی بالقوه برای واکسن جایگزین در برابر انواع اپیدمی ویروس تب برفکی باشد.

واژه‌های کلیدی: تست‌های تشخیصی سرولوژی، اپی توپ، تب برفکی، پپتید نوترکیب هفت اپی توپی، واکسن‌های پپتیدهای سنتزی

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