

Sequence Analysis of the Foot and Mouth Disease Virus Type O/IRN/2007 *VP1* Gene from Iranian isolate

(foot and mouth disease virus / nucleotide sequence / *VP1* gene / alignment / phylogenetic tree)

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Abstract. The foot and mouth disease virus (FMDV) causes a vesicular and contagious disease of cloven-hoofed animals. In this study, the virus was isolated from vesicles of the infected cattle using cell culture and serotyped by ELISA test. The extracted RNA from the infected cells was reverse transcribed and amplified using *VP1* gene-specific primer pairs by means of one-step RT-PCR. The purified *VP1* gene was sub-cloned into the unique *KpnI* and *BamHI* cloning sites of the pcDNA3.1+ vector. The DH5 α strain of *E. coli* was transformed by the vector. The sequences of sub-cloned FMDV type O/IRN/2007 *VP1* were aligned with FMDV type O/UKG/2001 *VP1* using MegAlign software. Nucleotide sequence comparisons were made using the BLAST software available from the NCBI website. The amino acid sequences of three sub-cloned FMDV type O/IRN/2007 *VP1* were also aligned with three other similar sequences using MegAlign software. Nineteen of the most similar *VP1* nucleotide sequences (by BLASTN program), FMDV O/IRN/2007 *VP1* sequence, twenty isolates of FMDV-O *VP1* in Iran and eight topotypes of FMDV type O were aligned by Mega5 to create a FMDV-O *VP1*-based sequence similarity tree. The nucleotide sequence comparison indicated that FMDV O/IRN/2007 *VP1* had the greatest nucleotide sequence similarity to the *VP1* gene of FMDV O1/Manisa/Turkey/69 (99 %), FMDV O1/Manisa/Netherlands (98 %) and FMDV O1/Manisa/iso87/Turkey (98 %).

It was also observed that the highest identity between FMDV O/IRN/2007 *VP1* sequence and other nucleotide sequences of FMDV type O *VP1* genes isolated in Iran during 1997–2004 was about 91 %.

Introduction

Foot and mouth disease (FMD) is a vesicular and highly contagious disease of cloven-hoofed animals, but rarely infects human (Brown, 2001). Severe economic loss was evidenced during the FMD epidemic in the United Kingdom in 2001 (Qian et al., 2003). The relevant causative agent is foot and mouth disease virus (FMDV), an aphthovirus of the Picornaviridae family. The virus contains a single-stranded positive-sense RNA genome about 7.2–8.4 kb in size. Seven major serotypes (A, Asia-1, C, O, SAT-1, SAT-2, and SAT-3) are known, and hundreds of isolates have already been described and partially sequenced (Qian et al., 2003). A particular genetic lineage of FMDV serotype O, PanAsia strain, is responsible for an explosive pandemic in Asia (OIE, 2009). Stamping out usually leads to more rapid eradication of FMD than vaccination, but in the short term is very expensive and resource exhaustive (Barnett et al., 2004). Apart from strictly veterinary considerations, public perceptions and environmental concerns must be taken into account. Combined strategies are very useful in many circumstances. On the other hand, continuous disease outbreaks are reported in endemic countries, due to current socio-economic conditions and geographical situation. Most of the disease outbreaks in FMD endemic countries like Iran are due to the serotype O followed by Asia-1 and A. Vaccination in Iran is carried out using trivalent inactivated viral vaccines (Barnett et al., 2004).

In 2007, a new type O, the type O PanAsia-2, was detected in the Middle East. This new strain probably originated from a strain circulating in India in 2001. It subsequently became pandemic in other countries such as Pakistan, Iran, Jordan, Turkey, the Palestinian Autonomous Territories, UAE, Kuwait, Bahrain, KSA, probably Lebanon and Egypt. It was responsible for high mortalities among lambs and calves during the winter,

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Abbreviations: CPE – cytopathic effect, FCS – foetal calf serum, FMD – foot and mouth disease, FMDV – foot and mouth disease virus, NCBI – National Center for Biotechnology Information, VNT – virus neutralization tests.

notably in 2007. A good immune response to this strain was produced by vaccination with type O Manisa (OIE, 2009).

Six FMDV type O isolates (O IRN 26/2007; O IRN 30/2007; O BAR 2/2008; O KUW 4/2008; O YEM 4/2006 and O YEM 29/2006) from Iran, Bahrain, Kuwait and Yemen were collected between 2006 and 2008 and characterized by two-dimensional virus neutralization tests (VNT). The results showed that most of these isolates were antigenically matched with O1 Manisa vaccine strains. The FMDV type O PanAsia-2 strain had dominated in the Middle East (Pakistan, Iran, Turkey, and Saudi Arabia) from July until September 2008 (OIE, 2009).

In this study the FMDV type O was isolated from cattle vesicles in 2007 in Iran. The *VP1* gene of FMDV type O/IRN/2007 was amplified using a pair of the specific primers. It was sub-cloned into pcDNA3.1+ in order to prepare a DNA vaccine against the virus. The similarity of the above-mentioned virus sequence with other subtypes of FMDV type O available at the NCBI website was also evaluated in the present study.

Material and Methods

Virus isolation

The epithelial cells of vesicles from three cattle displaying FMD clinical symptoms were collected around Tehran-Ray in 2007. The viruses were isolated in the Razi Vaccine and Serum Research Institute of Karaj, Iran. Pig kidney cells (IBRS₂) were grown in Earl's Modified Eagle's Medium (EMEM, Gibco, Carlsbad, CA) containing 10% (v/v) foetal calf serum (FCS, Gibco) at 37 °C and used for virus cultivation (Qian et al., 2003).

The inoculated cells showing considerable cytopathic effect (CPE) on the first passage were collected by freezing and thawing for preparation of FMDV antigen for the ELISA test and RNA extraction.

Serotyping of FMDV antigen

Serotyping of the isolated FMDV antigens was done using polyclonal antibodies against the seven serotypes (FMD Diagnostic Kit, Pirbright, UK). Two-dimensional neutralization test was used to characterize the relationship between field isolates of a particular serotype and a vaccine strain (Booth et al., 1978). The relationship between the field and the vaccine strains was expressed as an 'r' value, which was calculated according to the following equation: "Reciprocal log₁₀ of (heterologous titre – homologous titre)" (Barnett et al., 2004).

RNA extraction

Infected cells in the first passage were lysed by repeated freezing and thawing and the cell debris was removed by centrifugation for 12 min at 1,000 g. The total RNA was extracted directly from the supernatant using RNeasy Mini Kit (Qiagen, Hilden, Germany) according

to the manufacturer's instructions. The concentration of the total RNA was measured by Nanodrop (ND-1000, Thermo Scientific, Willmington, DE).

Primer design for VP1 gene and RT-PCR

The primer for amplification and cloning of the *VP1* gene was designed using the FMDV O/2001/UKG published sequence (Accession number: DQ165019.1) belonging to O PanAsia. The sequences of forward and reverse primers were designed by AlleleID6 software. The sequences of specific forward and reverse primers are F: 5'-CGGGGTACCACCATGGTTGACGCTCGCACGCAG-3', R: 5'-CGCGGATCCCTATTACAGGTCAAAGTTCAAAAGC-3', respectively. There were *KpnI* and *BamHI* sequences and three overhang nucleotides at the first of forward and reverse primers, respectively. The forward primer contained the kozak consensus sequence and start codon, and it started 21 nucleotides before the *VP1* sequence. The reverse primer contained two stop codons and ended 12 nucleotides after the *VP1* sequence. The extracted RNA was reverse transcribed and amplified using the *VP1* gene-specific primer pair AccuPower one-step RT-PCR kit (Bioneer, Daejeon, Korea). The PCR product 699 bp in size was purified by DNA gel extraction kit (Fermentas, Burlington, Canada).

Cloning and sequencing

The purified *VP1* gene was sub-cloned into the unique *KpnI* and *BamHI* cloning sites of the pcDNA3.1+ vector (Invitrogen, Carlsbad, CA) to construct the *VP1* gene cassette as used previously (Ke et al., 2009). The DH5 α strain of *Escherichia coli* was transformed with the vector using the heat-shock and CaCl₂ method. Three positive clones were confirmed by restriction enzyme digestion and colony PCR. The confirmed clones were sequenced using pcDNA3.1+ universal primer (T7 Forward) (Kim et al., 2006; Nagarajan et al., 2008).

Alignment and comparison of nucleotide and amino acid sequences

The FMDV type O/UKG/2001 *VP1* sequence (Accession number: DQ165019.1) was obtained from the National Center for Biotechnology Information (NCBI, Bethesda, MD) website. Three sub-cloned FMDV type O/IRN/2007 *VP1* sequences and the FMDV type O/UKG/2001 *VP1* sequence were aligned using MegAlign (DNASTAR, Madison, WI) software. Sequence comparisons were made by the use of the BLAST software available from the NCBI website using default search parameters. The amino acid sequences of the sub-cloned FMDV type O/IRN/2007 *VP1* and three most similar sequences were also aligned using MegAlign software as used previously (Kurz et al., 1981; Mohapatra et al., 2004; Jinding et al., 2006; Mingqiu et al., 2008).

Phylogenetic analysis

The most similar *VP1* nucleotide sequences were selected by the BLASTN program available from the

NCBI website for analysis of the FMDV O/IRN/1/2007 VP1 sequence and they were aligned by Molecular Evolutionary Genetics Analysis, version 5 (Mega5).

Nineteen of the above-mentioned VP1 nucleotide sequences, FMDV O/IRN/1/2007 VP1 sequence, 20 isolates of FMDV-O VP1 in Iran from 1997–2004 and eight topotypes of FMDV type O defined by Knowles and Samuel (2003) were aligned by Mega5 and used to create a FMDV-O VP1-based sequence similarity tree using the neighbour-joining algorithm (Fig. 1).

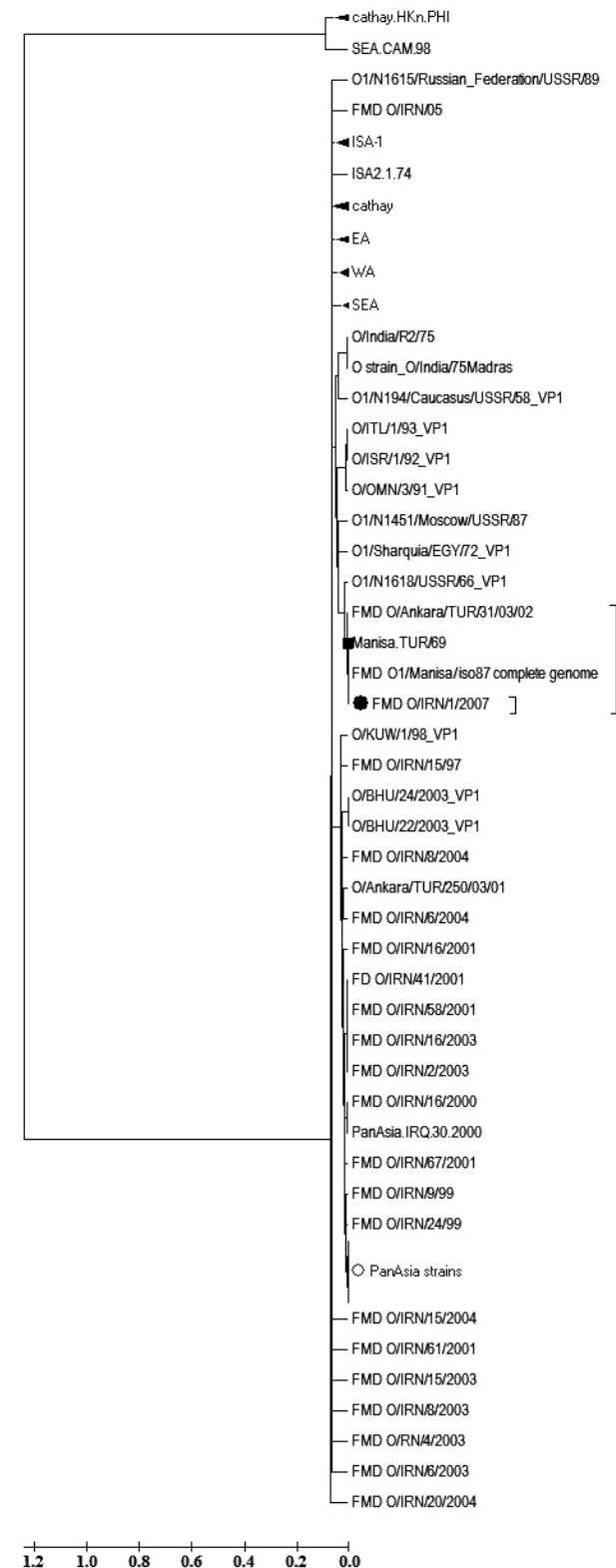


Fig. 1. The similarity tree of VP1 FMDV type O/IRN/2007 with the VP1 nucleotide sequences isolated in Iran in 1997–2004, 19 of the most similar VP1 nucleotide sequences (by BLASTN program) and eight topotypes of FMDV type O by Mega5 software

Results

Serotyping of the isolated FMDV antigen was done by ELISA which subsequently showed serotype O. Furthermore, the ‘r’ value of isolated FMDV type O/IRN/2007 to the vaccine strain for O Shabestar (former vaccine strain) and O967 Markazi (current vaccine strain) were 0.81 and 0.88, respectively.

Nucleotide sequence of FMDV O/IRN/1/2007 VP1

There are 672 nucleotides and 224 amino acid residues in the VP1 coding region. Fig. 2 shows the nucleotide and amino acid sequences. The nucleotide sequence data was deposited in Gen Bank database under the accession number JF288761.

Nucleotide sequence comparison

A nucleotide sequence comparison drawn by the BLASTN program with default search parameters indicated that FMDV O/IRN/1/2007 VP1 had the greatest sequence similarity to FMDV serotype O Manisa. The nine most similar sequences to the nucleotide and amino acid sequences of FMDV O/IRN/1/2007 VP1 were identified using the Nucleotide BLAST and TBLASTX programs and are shown in Table 1. Compared with these nine isolates, the nucleotide identity ranged from 89 % to 99 % and the amino acid identity ranged from 95 % to 100 %. It is most probable from the analysis of the VP1 coding region and its product that FMDV O/IRN/1/2007 belongs to serotype O (Knowles, 2003).

The phylogenetic tree is shown in Fig. 1. From this analysis, the FMDV O/IRN/1/2007 VP1 sequence shared the greatest similarity at the nucleotide level with O1/Manisa/Turkey/69, O1/Manisa/Netherlands and O1/Manisa/iso87/Turkey. The nucleotide sequence identity

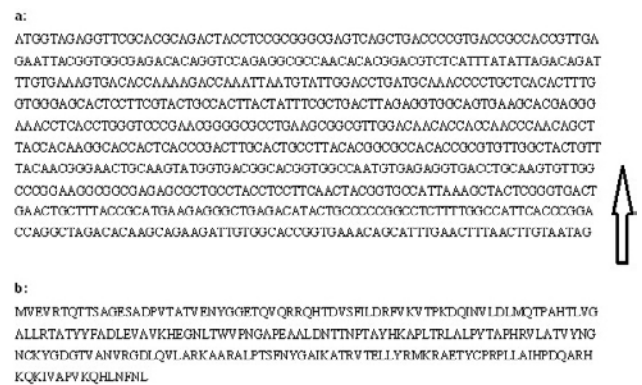


Fig. 2. The nucleotide (A) and the translated amino acid (B) sequences of FMDV O/IRN/1/2007 VP1 (clones 1 and 3)

Table 1. Similarity between FMDV O/IRN/2007 VP1 and the most closely related isolates obtained from sequence comparison

Gen Bank Accession No.	Serotype	Strain	Location	Nucleotide sequence similarity (%)	Amino acid sequence similarity (Identities %)
FN594747.1	O	O1/Manisa	Netherlands	98	99
AJ251477.1	O	O1/Manisa/Turkey/69	Turkey	99	100
AY593823.1	O	O1/Manisa/iso87	Turkey	98	98
DQ296524.1	O	O/Ankara/TUR/31/03/02	Turkey	97	96
AJ004678.1	O	O1/N1618/USSR/66	Russia	96	96
AY593824.1	O	O1/SKR/iso85	South Korea	91	99
AY145879.1	O	O/India/75/Madras	India	91	95
DQ164905.1	O	O/KUW/1/98	Kuwait	91	95
AY312587.1	O	O/SKR/2000	South Korea	89	99

Table 2. The nucleotide sequence similarity of FMDV type O/IRN/2007 VP1 gene with the VP1 nucleotide sequences which were isolated in Iran

Gen Bank Accession No.	Serotype	Strain	Location	Nucleotide sequence similarity (%)
DQ165056.1	O	O/IRN/20/2004	Iran	89
DQ165055.1	O	O/IRN/15/2004	Iran	89
DQ165054.1	O	O/IRN/8/2004	Iran	91
DQ165053.1	O	O/IRN/6/2004	Iran	90
DQ165052.1	O	O/IRN/16/2003	Iran	90
DQ165051.1	O	O/IRN/8/2003	Iran	90
DQ165050.1	O	O/IRN/6/2003	Iran	90
DQ165049.1	O	O/IRN/4/2003	Iran	90
DQ165048.1	O	O/IRN/2/2003	Iran	91
DQ164898.1	O	O/IRN/15/2003	Iran	90
DQ164897.1	O	O/IRN/67/2001	Iran	91
DQ164896.1	O	O/IRN/61/2001	Iran	89
DQ164895.1	O	O/IRN/58/2001	Iran	90
DQ164894.1	O	O/IRN/41/2001	Iran	90
DQ164893.1	O	O/IRN/16/2001	Iran	90
AJ318840.1	O	O/IRN/16/2000	Iran	90
AJ318839.1	O	O/IRN/24/99	Iran	89
AJ318838.1	O	O/IRN/9/99	Iran	90
AJ318837.1	O	O/IRN/15/97	Iran	89

of VP1 FMDV type O/IRN/2007 with the VP1 nucleotide sequences that had been isolated in Iran in 1997–2004 is shown in Table 2.

The amino acid sequences of VP1 for O/IRN/2007, FN594747.1 (O1/Manisa/Netherlands), AJ251477.1 (O1/Manisa/Turkey/96), AY593823.1 (O1/Manisa/iso87) and O/UKG/2001 (DQ165019.1) were compared by MegAlign software. The result is shown in Fig. 3. The result of amino acid sequence alignment showed the highest similarities among VP1 for O/IRN/2007 (three clones), FN594747.1 (O1/Manisa/Netherlands), AJ251477.1 (O1/Manisa/Turkey/96) and AY593823.1 (O1/Manisa/iso87). However, the major differences for VP1 of FMDV type O/UKG/2001 (DQ165019.1) were in the position 138–142 (Table 3).

Discussion

To treat FMD, inactivated vaccines are used, but vaccine strains must be carefully matched to prevailing field virus strains to induce a satisfactory level of protection; vaccination must cover a level of at least 80% effectiveness (Hassanein et al., 2011). Due to the multiple serotypes of FMDV in circulation, identification of

the serotype affecting any one region is required in order to select the most appropriate antigens for inclusion in the vaccine preparation. The most important immunogenic site of FMDV is the VP1 surface antigen encoded by the 1D region (Qian et al., 2003).

Mahravani et al (2007) reported the sequencing results of type O isolated in Iran from 2005 until 2006, showing a close genetic relationship between the field isolates and Iranian vaccine strain (Shabestar strain) (Mahravani et al., 2007).

The Middle East represents a very complex epidemiological situation. FMDV type O is endemic in all countries of the region, and the trading relationships, both legal and illegal, are reflected in the movement of individual strains. Many unreported outbreaks occur and attempts to limit the spread of disease are generally unsuccessful. Outbreaks of FMD caused by type O in Bulgaria (1991, 1993 and 1996), Italy (1993), Greece (1994 and 1996) and European Turkey (1995–96) were genetically closely related to viruses circulating in the Middle East (Samuel and Knowles, 2001).

Two FMDV type O isolates (O IRN 26/2007; O IRN 30/2007) from Iran collected in 2006 were further characterized by two-dimensional virus neutralization tests.

TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	Majority
TTSAGESADPVTATVENYGGGETQVQRRQ.TDVSFILDRFV	Consensus #1
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O.UKG.2001
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O1.Manisa/iso87
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O1.Manisa/Netherlands
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O1.Manisa/Turkey69
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O.2007.clone1
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O.2007.clone2
1 TTSAGESADPVTATVENYGGGETQVQRRQHTDVSFILDRFV	FMDV.VP1.O.2007.clone3
KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	Majority
KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	Consensus #1
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O.UKG.2001
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O1.Manisa/iso87
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O1.Manisa/Netherlands
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O1.Manisa/Turkey69
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O.2007.clone1
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O.2007.clone2
41 KVTPKDQINVLDLMQTPAHTLVGALLRTATYFFADLEVAV	FMDV.VP1.O.2007.clone3
KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	Majority
KHEG.LTW.PNGAPE.A.DNTTNPTAYHKAPLTRLALPYT	Consensus #1
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O.UKG.2001
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O1.Manisa/iso87
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O1.Manisa/Netherlands
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O1.Manisa/Turkey69
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O.2007.clone1
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O.2007.clone2
81 KHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT	FMDV.VP1.O.2007.clone3
APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	Majority
APHRVLATVYNGN.KYG...V.NVRGDLQVLA.KAAR.LP	Consensus #1
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O.UKG.2001
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O1.Manisa/iso87
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O1.Manisa/Netherlands
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O1.Manisa/Turkey69
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O.2007.clone1
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O.2007.clone2
121 APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALP	FMDV.VP1.O.2007.clone3
TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	Majority
TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	Consensus #1
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O.UKG.2001
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O1.Manisa/iso87
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O1.Manisa/Netherlands
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O1.Manisa/Turkey69
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O.2007.clone1
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O.2007.clone2
161 TSFNYGAIKATRVTELLYRMKRAETYCPRLLAIHPDQAR	FMDV.VP1.O.2007.clone3
HKQKIVAPVKQLL	Majority
HKQKIVAPVKQLL	Consensus #1
201 HKQKIVAPVKQLL	FMDV.VP1.O.UKG.2001
201 HKQKIVAPVKQLL	FMDV.VP1.O1.Manisa/iso87
201 HKQKIVAPVKQLL	FMDV.VP1.O1.Manisa/Netherlands
201 HKQKIVAPVKQLL	FMDV.VP1.O1.Manisa/Turkey69
201 HKQKIVAPVKQLL	FMDV.VP1.O.2007.clone1
201 HKQKIVAPVKQLL	FMDV.VP1.O.2007.clone2
201 HKQKIVAPVKQLL	FMDV.VP1.O.2007.clone3

Fig. 3. Amino acid alignment report of VP1 for FMDV O/IRN/2007 (three positive clones), FMDV O1/Manisa/Netherlands (FN594747.1), FMDV O1/Manisa/Turkey/69 (AJ251477.1), FMDV O1/Manisa/iso87 (AY593823.1) and FMDV O/UKG/2001 (DQ165019.1) using MegAlign software (ClustalW)

The results showed that these isolates matched the O1 Manisa vaccine strains in antigenic diversity (OIE, 2009).

In the present study the nucleotide sequence comparison made by the BLASTN program with default search parameters indicated that FMDV O/IRN/1/2007 VP1 had the greatest sequence similarity to the VP1 gene of FMDV O1/Manisa/Turkey/69 (99 %), FMDV O1/Manisa/Netherlands (98 %) and FMDV O1/Manisa/iso87 (98 %). The highest level of nucleotide sequence identity of FMDV O/IRN/1/2007 VP1 with the nucleotide sequences of FMDV type O VP1 genes isolated in Iran was 91 %. Nucleotide sequences of FMDV O/IRN/1/2007 VP1 clone 1 and clone 3 were exactly the same.

The tripeptide RGD at the 145-147 amino acid position was conserved for VP1 of FMDV O/IRN/2007 and all O1 Manisa strains (FN594747.1, AJ251477.1, and AY593823.1) included in the analysis and for O/UKG/2001 (DQ165019.1). The GH loop, amino acid positions 140-160, was completely conserved across the VP1 of O/IRN/2007 (three clones) and O1 Manisa strains, but differed from O/UKG/2001 in four amino acid positions including 138, 139, 140 and 142 (Carrillo et al., 2005; Knowles and Samuel, 2005; Parlak et al., 2007; Ayelet, 2009). The O/IRN/2007 and O1 Manisa viruses had aspartic acid, glycine, threonine and alanine in these positions, whereas the O/UKG/2001 virus had glutamic acid, serine, proline and threonine in these positions. All of the results indicate that a good immune response may be induced against FMDV O/IRN/2007 by type O Manisa vaccination.

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