Original Article

Molecular detection, genetic diversity, and phylogenetic analysis of foot-and-mouth disease virus (FMDV) type O in Iran during 2015-2016

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Abstract

Background: The major challenge of foot-and-mouth disease (FMD) control is attributed to the rapid mutations in the FMDV RNA genome, resulting in continuous antigenic changes of circulating strains. Despite widespread vaccination of livestock populations, the incidence of the FMDV serotype O outbreaks in Iran during 2015-2016 raised concerns about the emergence of new strains. **Aims:** The aim of this study is the genetic and antigenic evaluation of FMDV type O isolates from different outbreak areas including Alborz, Tehran, Isfahan, Markazi, Zahedan, and Qom provinces. **Methods:** For this purpose, 71 FMD-infected samples were collected from six provinces of Iran, of which 12 serotype O positive were selected for genetic analysis. **Results:** All samples were in ME-SA topotypes/OPanAsia2 lineage, and the overall mean of genetic diversities at the 1D gene level was about 5% between the sequences. Blasting 1D gene sequences of isolated viruses showed more than 90% genetic identity with sequences registered from neighboring countries; therefore, it could be concluded that they had a common origin. Six isolates showed the highest genetic diversity (6% to 11%) with the OPanAsia2 vaccine strain (JN676146), which three of them (Qom, Alborz, and Zahedan isolates), had less than 30% antigenic homology with the OPanAsia2 virus (JN676146). **Conclusion:** Results of this study suggested OPanAsia2 vaccine had no enough coverage with some circulating strains in outbreak areas in Qom, Alborz, and Zahedan provinces, and the necessity of OPanAsia2 replacement with a new vaccine strain in Iran.

Key words: Foot-and-Mouth disease virus, Genetic variation, Phylogeny

Introduction

Foot-and-Mouth disease (FMD) is a highly contagious vesicular disease that affects domestic and wild cloven-hoofed mammals. FMD is a transboundary animal disease (TAD) that causes massive economic losses to the livestock industry, and it is a significant barrier to guaranteeing animal health and welfare (OIE, 2015). Although many countries were recognized as FMD-free zones by the World Organization for Animal Health (OIE), many areas are still endemic for the disease, especially in Africa and Asia continents. The etiological agent of the disease is the foot-and-mouth disease virus (FMDV), which belongs to the *Aphthovirus* genus of the Picornaviridae family (Zell *et al*., 2017).

FMDV, with a diameter of about 25-30 nm, is composed of an 8.4-kilobase, positive-sense, single-stranded RNA genome surrounded by a non-enveloped icosahedral capsid (Fig. 1) (Jamal and Belsham, 2013). The genome contains a single large open reading frame, which encodes a large polyprotein that processes to primary products: L^{pro}, P1-2A, P2, and P3. P1-2A is a capsid precursor whose cleavage by the 3C protease ($3C^{pro}$) at three junctions produces four structural proteins (VP1-VP4) and 2A. P2 and P3 regions encode non-structural proteins: 2B, 2C, 3A, 3B1-3, or Vpg, $3C^{pro}$, and $3D^{pol}$, for virus replication and pathogenesis. The two ends of the genome consist of a large 5´ UTR region, which contains regulatory parts such as IRES, and a small 3´ UTR, ending in a poly-A tail (Fig. 1) (Carrillo *et al*., 2005).

As with the most rapidly evolving RNA viruses, the FMD genome is prone to mutation because of the RNA polymerase's lack of proofreading capability during viral replication. Additionally, immunity-induced selective pressure following infection or vaccination, and

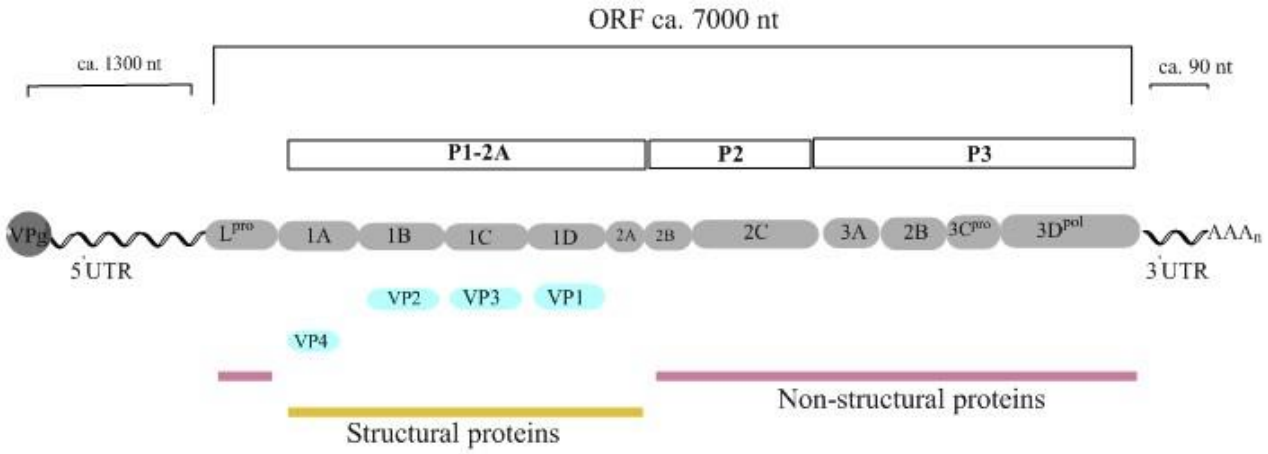

Fig. 1: The FMDV RNA genome contains a single large open reading frame (ca. 7000 nt), which encodes a large polyprotein being processed into primary products: L^{pro}, P1-2A, P2, and P3 in a protease independent break. Cleavage of P1-2A by the 3C protease ($3C^{pro}$) is essential for producing four capsid proteins (VP1-VP4) and 2A. P2 and P3 regions produce non-structural proteins: 2B, 2C, 3A, 3B1-3, or Vpg, $3C^{pro}$, $3D^{pol}$ that are involved in virus replication and pathogenesis. The each side of the genome is included in a large 5´ UTR region (ca. 1300 nt) and a small 3´ UTR (ca. 90 nt), ending in a poly-A tail

recombination between FMDV serotypes also contribute to genetic changes in the viral genome that lead to the predominance of escape mutants and the formation of quasi-species populations (Domingo *et al*., 2003; Aiewsakun *et al*., 2020).

There are seven distinct serotypes of FMDV in circulation worldwide: O, A, C, Asia1, and South African Territories "SAT 1, 2, and 3"; each diverges into genetically different strains, lineages, and specific geographical topotypes (Brito *et al*., 2017). There are no specific pathognomic signs related to any serotype, and infection/vaccination with one serotype does not provide cross-immunity protection against other serotypes or even different strains in one serotype. Therefore, regular monitoring of the compatibility of the vaccine strains with the circulating variants is critical for successful containment programs (Mateu *et al*., 1994). Fast and reliable approaches for genetic and antigenic characterization of circulating strains such as the virus neutralization test (VNT) and sequencing of the 1D gene critically enable the vaccine efficiency or genetic dynamics assessment in circulating variants (Samuel and Knowles, 2001; Mattion *et al*., 2009; Knowles *et al*., 2016). The virus neutralization test is an *in vitro* laboratory method to measure the titer of the neutralizing antibodies, and it is considered a gold standard for the assessment of protective antibodies. Moreover, genetic drifts in FMDV 1D gene lead to the development of new subtypes or strains of FMDV; therefore, sequencing and phylogenetic analysis of the 1D gene provides valuable insight into the FMDV origin of outbreak or mapping of the virus spillover (OIE, 2018).

FMD is considered one of the important endemic diseases in Iran which threaten the livestock economy. In Iran, like other Asian countries, type O is the most important and dominant FMDV in outbreaks. During past years FMDV type O strains in the vaccine were changed several times in Iran. These changes were made to induce better immunity and increase vaccine effectiveness (Unpublished data). The first FMDV was identified in 1955 and registered under the name of O1 (Firouzi Bandpay *et al*., 1984). In 2006, a large outbreak of the disease occurred with serotype O in Iran and other countries in the Middle East and Central Asia which was named OPanAsia (JQ321837.1), and was used in the vaccine formulation until 2009. In 2010, another large-scale outbreak of FMDV type O occurred in Iran, which was simultaneously observed in other neighboring countries. This newly isolated virus was named OPanAsia2 (JN676146) which replaced OPanAsia (JQ321837.1) in the vaccine production (unofficial report). OPanAsia2 was successfully used in the vaccination campaigns until 2016 when the disease began to spread around Qom province and gradually scattered to the other parts of Iran. FMD spill despite widespread livestock vaccination raise new concern about vaccine coverage in 2016. The aim of this study is the genetic and antigenic evaluation of FMDV type O isolates from different outbreak areas including Alborz, Tehran, Isfahan, Markazi, Zahedan, and Qom provinces which allows us to assess the efficiency of the current vaccine in Iran.

Materials and Methods

Sample collection and preparation

Following the disease outbreak during 2015-2016, 71 samples were collected from FMD outbreak areas in Alborz, Tehran, Isfahan, Markazi, Zahedan, and Qom provinces (Table 1). These samples were collected from the oral epithelium and hoof tissues of cattle, sheep, and goats with FMD-specific clinical signs and then sent to the FMD reference lab of the Razi Vaccine and Serum Research Institute (RVSRI). For sample preparation, about 1 to 2 g of each tissue were homogenized and suspended in a 5 ml cell culture medium. The suspensions were centrifuged at 3000 × g for 20 min, and

then the supernatants were stored at -20°C for further analysis (OIE, 2008). The presence of FMDV antigen was detected in all tissue homogenates by the antigen detection and serotyping ELISA kit (IZSLER, Brescia, Italy) according to the manufacturer's instructions.

Table 1: The total suspected and positive FMD clinical samples were received from each province in this study

Provinces name	Number of received sample	Detected FMDV type O by ELISA
Alborz	13	5
Markazi	9	3
Isfahan	14	4
Qom	13	5
Tehran	12	3
Zahedan	10	4
total	71	24

Samples were collected from all provinces with disease outbreak during 2015-2016. FMDV: Foot-and-Mouth disease virus, and ELISA: Enzyme-Linked immunosorbent assay

Virus isolation

The IBR-S2 cell line was used for virus isolation. For this purpose, the IBR-S2 cell culture was prepared by incubation for 48 h at 37°C in RPMI1640 medium (Sigma) supplemented with FBS 5%, penicillin 100 IU/L, and streptomycin 100 mg/L. Upon the IBR-S2 cells reaching the flask at more than 80% confluency, the cells were inoculated by the prepared clinical samples. When 90% of the infected cells were detached from the flask surface due to the cytopathic effect, they were frozen at -70°C until used for RNA extraction.

RNA extraction

Total RNA was extracted from the supernatant of the infected IBR-S2 cells using TRIzol® (Invitrogen, USA), according to the manufacturer's instructions. The extracted viral RNA was quantified by a NanoDrop spectrophotometer (NanoDrop® ND-1000) at wave-lengths 230, 260, and 280 nm.

In addition to the field samples, viral RNA was also extracted from two vaccine strains including OPanAsia (JQ321837.1) and OPanAsia2 (JN676146) using a high-purification viral extraction kit (Roche, Germany).

RT-PCR and 1D gene sequencing

Viral RNAs were reverse-transcribed into the cDNA using Prime RT Premix (2x) kit based on the manufacturer's instruction (Genet Bio, South Korea). In brief, after mixing recommended materials in the final volume of 20 µL, the mixture was incubated for 1 h at 37°C for cDNA synthesis and incubated for 5 min at 95°C to inactivate reverse transcriptase. In the next step, the cDNA was subjected to the PCR reaction (2X Pfu PCR Master Mix, biotech rabbit GmbH, Germany). O-type specific primers were used for the 1D gene replication Table 2. The primer set bound to the upstream (1C) and downstream regions (2B) of the 1D gene to amplify its complete sequence. PCR program included denaturation at 95°C for 5 followed by 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C extension time for 45.

PCR amplicons were visualized by electrophoresis in 1.5% agarose gel, purified by High Pure PCR Product Purification kit (Sigma-Aldrich), and sent for the sequencing (Macrogen Inc., South Korea).

After receiving the sequences of the 1D gene, they were trimmed by Chromas package (2.6.6) and then assembled using Contig Assembly Program (CAP). The final consensus of the 1D gene of the field samples was compared to any relevant sequence using Nucleotide Biological Local Alignment Tool (BLAST) available in the NCBI database. FMD viruses that had the highest genetic relationship (expected value = 0) with the 1D gene of the field samples were identified and selected for the bioinformatics evaluations.

Phylogenetic analysis

The 1D gene final sequences of the filed samples plus two vaccine strains (OPanAsia and OPanAsia2) were aligned using the BioEdit tool v.7.2.5 (BioEdit; RRID: SCR_007361). In the next step for evaluating the 1D gene variability among the strains, the sequences were imported into the MEGA 11.0 package. Additionally, seven prototypes of the Middle East-South Asia (ME-SA) topotype include sub-lineages of ANT-10, BAL-09 and FAR-09, and lineages of Ind2001a, Ind2001b, Ind2001c and Ind2001d from the World Reference Laboratory of FMD (WRL-FMD) (http://www.wrlfmd.org/fmd_genotyping/prototypes.htm) were used for phylogenetic tree generation. FMD O Rey virus (AY593834) was used as an outgroup in the phylogenetic analysis; this virus was isolated from the Ray region around Tehran in 1966 and is registered as the oldest Iranian FMD virus in the NCBI. The evolutionary divergence among the samples was inferred based on the Kimura method (Kimura 2-parameter nucleotide substitution model) and the phylogenetic tree was constructed using the neighbor-joining (NJ) method, as implemented in MEGA 11 (Tamura *et al*., 2021).

Preparation of reference antiserum

Two doses of a commercial polyvalent vaccine comprising inactivated Opanasia2, A05IR, and Asia1 viruses (RVSRI, Iran) were injected into a 10-month-old, seronegative bull to produce a reference antiserum. For this purpose, two doses of the vaccine were given to the

Table 2: Primer pair used for 1D gene in PCR assay

Primer name	Primer sequence (5´-3´)	Product length	Reference
O-1C564F	AATTACACATGGCAAGGCCGACGG	789 nt	Knowles *et al*. (2016)
NK72	GAAGGGCCCAGAGGGTTGGACTC		

calf 21 days apart, and the seroneutralization test was used to determine the blood antibody titer 28 days after the second dose injection (OIE, 2018).

Two-dimensional neutralization test (2D-VNT)

Antigenic relationship of field samples with OPanAsia2 (JN676146) virus was determined by 2D-VNT test according to the OIE manual (OIE, 2008). The antigenic relationship (r-value) of field viruses with vaccine virus (OPanAsia2) was calculated by the following formula:

The reciprocal reference serum neutralization titer against field viruses/reciprocal reference serum neutralization titer against OPanAsia2 virus

According to the standard guidelines, if the r-value between two viruses is calculated to be ≥30%, these viruses have antigenic coverage, otherwise they are antigenically distinct (Mattion *et al*., 2009; OIE, 2018).

Results

Molecular characterization of FMDV

In the present study, a total of 71 FMD-infected clinical isolates from the outbreak areas in Iran were serotyped using ELISA, of which 24 (34%) were identified as serotype O (Table 1 and Fig. 2). From serotype O isolates, 12 samples were subjected to PCR amplification of the 1D gene along with two vaccine strains (OPanAsia (JQ321837.1) and OPanAsia2 (JN676146)). After sequencing of amplicons and blasting of contigs in the NCBI database, the 1D gene final consensus of the field samples revealed the highest genetic relationship (more than 90% similarity) with 11 sequences from Iran and neighboring countries such as Pakistan (JX170757.1, KJ831720.1), Turkey (KJ831714.1), Iraq (KY412559.1), Saudi Arabia (KJ831701.1), and United Arab Emirates (KR149714.1) (Fig. 2).

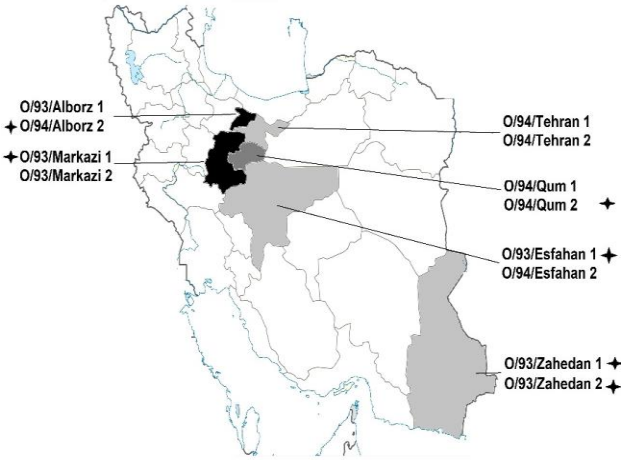

Fig. 2: The distribution of samples received from the foot-and-mouth disease outbreak area during 2015-2016. The name of samples related to each province are displayed. ✦ Indicate samples that used in antigenic relationship evaluation by 2D-VNT test

Phylogenetic and antigenic relationship evaluation

Total of 33 1D gene sequences were subjected to distance-based phylogenetic tree construction: 12 nucleotide sequences from outbreak regions, two nucleotide sequences belonging to OPanAsia (JQ321837.1) and OPanAsia2 (JN676146) vaccine strains, nucleotide sequences of the seven prototypes of the ME-SA topotype (registered at WRL-FMD), the first 11 hits of BLAST results of the field samples, and FMD O Rey virus (AY593834) as an outgroup were used in the tree construction (Fig. 3).

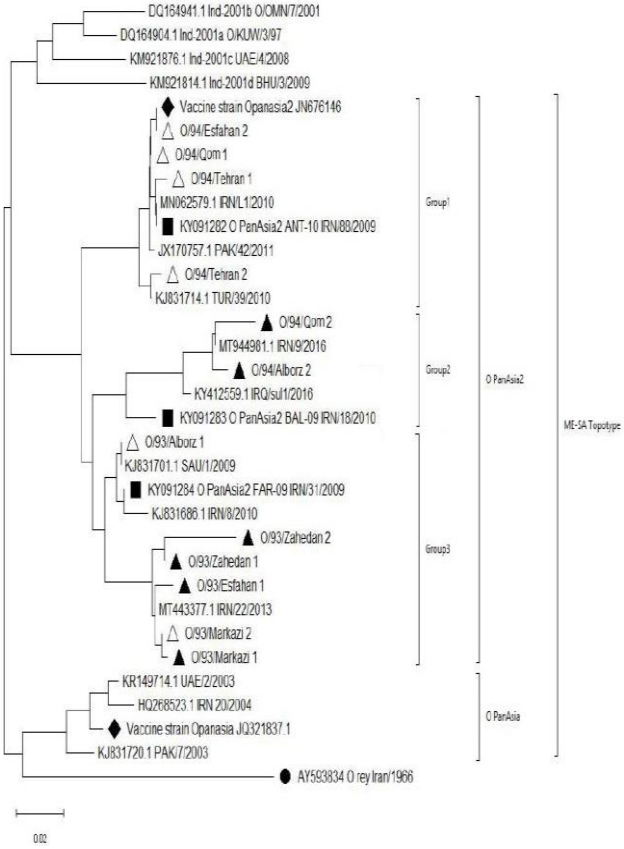

Fig. 3: Phylogenetic tree displays genetic relationship among FMDV type O isolated in 2015-2016 outbreak in Iran. This analysis involved 33 nucleotide sequences. The evolutionary history was inferred using neighbor-joining (NJ) method, tested with 500 bootstrap replicates and a threshold score of 70% in MEGA version 11. The evolutionary distances were computed using the Kimura 2-parameter method in the units of the number of base substitutions per site. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). ▲/△ Denote field samples that subjected or not subjected to 2D-VNT test respectively, ◆ Indicates vaccine strain, ■ Indicates sublineages that belong to lineage Opanasai2, and ● Marks outgroup

Genetic diversities within groups and between lineages of ME-SA topotype are presented in Table 3. Genetic analysis of clinical samples of infected animals showed that all of them belonged to the ME-SA

Table 3: Estimates of average evolutionary divergence over sequence pairs within groups and between groups

lineage		Within group	Between group		Within lineage	Between lineage	
			Group 1	Group 2		OPanAsia2	OPanAsia
OPanAsia2	Group 1	0.01			0.07		
	Group 2	0.03	0.09				
	Group 3	0.02	0.07	0.08			
OPanAsia					0.03	0.10	
Ind-2001a						0.11	0.08
Ind-2001b						0.12	0.09
Ind-2001c						0.11	0.09
Ind-2001d						0.13	0.12
Outgroup						0.17	0.13

Groups: OPanAsia, OPanAsia2, Ind-2001a, Ind-2001b, Ind-2001c, Ind-2001d lineages, and Outgroup (i.e. O Rey virus). Sequences in OPanAsia2 lineage were divided into three groups according to genetic diversity less than 3% in each group. Analyses were conducted using the Kimura 2-parameter model (Kimura, 1980). There were a total of 633 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021)

Table 4: The antigenic relationship (r-value) of FMDV strains obtained from the two-dimensional virus neutralization test (2D-VNT)

	O/94/Qom_2	O/94/Alborz_2	O/93/Zahedan_2	O/93/Zahedan1	O/91/Esfahan1	O/93/Markazi1
Serotype	O	O	O	O	O	*O*
Topotype	ME-SA	ME-SA	ME-SA	ME-SA	ME-SA	ME-SA
Lineage	PanAsia-2	PanAsia-2	PanAsia-2	PanAsia-2	PanAsia-2	PanAsia-2
r-value	0.27	0.28	0.28	0.33	0.41	0.48

r-value ≥30% indicates a significant antigenic difference between two relative viruses. ME-SA: Middle East-South Asia

topotype/OPanAsia2 lineage, and they showed about 10% genetic diversity with OPanAsia lineage (Table 3). Furthermore, the genetic diversity between genotypes is in the range of 10% to 15%. Sequences in the OPanAsia2 lineage were divided into three groups. The overall mean of genetic diversity was 5% between groups and was less than 3% in each group (Fig. 3 and Table 3).

Half of the field samples, which had more than 5% genetic diversity, were subjected to 2D-VNT to evaluate the antigenic relationship compared to the OPanAsia2 virus. These samples indicate genetic diversity as follows: O/94/Qom2 (11%), O/94/Alborz2 (10%), O/93/ Zahedan2 (11%), O/93/Zahedan1 (6%), O/91/Esfahan1 (8%), and O/93/Markazi1 (7%) (Table 3). Antigenic evaluations revealed that only three of these samples: O/94/Qom2, O/94/Alborz2, and O/93/Zahedan2, had no coverage with OPanAsia2 (r-value <0.3) (Table 4).

Discussion

Despite global efforts, FMDV pose serious animal health concerns in multiple major virus pools over three continental epidemiological clusters in Africa, Asia, and South America (Di Nardo *et al*., 2021). Molecular characterization of the VP1-encoding region is the basis of FMDV serotypes subdivision into different lineages and sub-lineages. Generally, sequence divergence of ~15% and 5% in the VP1-encoding sequences are arbitrary cut-offs for lineage and sub-lineage classification, respectively (Mohapatra *et al*., 2011). However, when the genetic diversity is between 5% and 15%, which would lead to antigenic drift of viral strain and vaccination failure, it is difficult to define the molecular phylogenetic category of a virus strain with accuracy. In these cases, attention to antigenic properties of circulating strains through the antigenic relationship (r-value) combined with phylogenetic analysis is a technical choice.

FMDV serotype O, the world's most prevalent serotype, is classified into 11 topotypes, one of which the ME-SA circulates in the Middle East and South Asia (Samuel and Knowles, 2001). ME-SA topotype includes O/PanAsia, O/PanAsia2, Ind2001a, Ind2001b, Ind2001c, and Ind2001d lineages with more than 7.5% genetic diversity. O/PanAsia is one of the important ME-SA lineages that has led to FMD outbreaks in Asia and even beyond the continent. This lineage first emerged in 1982 in India and gradually invaded other parts of the Asian continent, such as the Middle East, East, Southeast Asia, and even Europe since 1990 (Jamal and Belsham, 2013). Despite annual vaccination programs, a distinct variant known as FMDV O/PanAsia was responsible for several outbreaks in Iran since 2006. The emergence of vaccine-adapted mutants and uncontrolled animal trade play vital roles in the spillover of the disease and devastated economic loss in the country (Firouzi Bandpay *et al*., 1984).

The dominance of highly adapted viruses that spread widely within endemic regions was related to rare spillover to FMD-free countries similar to a pandemic with the serotype O lineage in 2001 (Knowles *et al*., 2005). Furthermore, co-circulating multiple FMDV lineage in the endemic regions is attributed to the inter-lineage recombination and may complicate the situation (Brito *et al*., 2018). Therefore, the genetic evaluation of field strains and improved knowledge about regional patterns of lineage or sub-lineage dominance in endemic areas could help to control the disease strategically by a timely vaccine strain selection.

Results of the present study showed that all field samples had less than 15% genetic diversity compared to

the OPanAsia2 vaccine strain (JN676146); therefore, field samples are the OPanAsia2 homologous strains (Fig. 3). Furthermore, the genetic diversity between genotypes is in the range of 10% to 15%. As previous research has shown, genetic diversity of 5% to 15% indicates antigenic drift of viral strain and vaccination failure (Mohapatra *et al*., 2011).

Antigenic evaluations of the isolates with more than 5% genetic diversity from Qom, Alborz, Zahedan, Esfahan, and Markazi provinces revealed that only three of these samples: O/94/Qom2, O/94/Alborz2, O/93/ Zahedan2, had no coverage with OPanAsia2 (r-value <0.3) (Table 3). Although there was a little antigenic difference between these isolates, it raised concerns about the virus's future predominance. FMD vaccine should be prepared using the most recent circulating strains. Therefore, the incidence of FMD infection in the vaccinated animals can be explained by a mismatch between the vaccine and the circulating strains and the need for vaccine strain substitution for maximum coverage.

However, the vaccine efficacy can be influenced by various factors; thus, the r-value <0.3 does not necessarily imply a complete lack of protection. Highly potent vaccines as well as delivering multiple doses administered at appropriate intervals can be used to compensate for a poor antigenic relationship between the vaccine strain and circulating strains isolates (Brehm *et al*., 2008). FMDV type A is the most variable virus among Eurasian serotypes (O and A), according to previous research (Mohapatra *et al*., 2011). However, the present findings displayed that genetic drifts in structural genes of FMDV type O, such as the 1D gene, led to the emergence of new subtypes or strains.

The livestock history of vaccination has influenced the prevalence of FMD disease in Iran along with numerous other variables, such as importing livestock from different farms, the transfer of nomadic livestock, the age distribution of the livestock population, and the less than standard 1 km distance between cultivating units (Bagheri Amiri *et al*., 2016). During FMD outbreaks cattle should be vaccinated three times a year. However, some studies have found a substantial link between the frequencies of FMD in highly endemic areas with some cattle populations that have not full compliance with FMD immunization (Kamalidoost *et al*., 2016; Ilbeigi *et al*., 2018).

The over 90% identity between the isolated viruses in Iran and the viruses registered from neighboring countries such as Pakistan, Turkey, Iraq, and Saudi Arabia in the GenBank database, indicates the simultaneous presence of OPanAsia2 lineage in the West of Asia. The use of genetically similar vaccine strains in several countries over a long time, plus extensive trans-regional animal trading led to the selection of a virus strain with different genetic and antigenic characteristics. Therefore, mutants can escape from the defense barrier of vaccinated livestock and target susceptible animals. Additionally, in countries with a large livestock population and no natural boundaries such as Iran, whose stringent trade restrictions are difficult to implement, controlling the invasion of emerging viruses is much more difficult.

Phylogenetic analysis of the circulating PanAsia2 lineage of FMDV O serotype in Punjab, Pakistan in 2014 has shown homology with previously characterized strains from Afghanistan, Iran, India, Nepal, and Bhutan (Kanwal *et al*., 2014). However, a variety of sub-lineages were emerging with O PanAsia2 FMDV in Pakistan, indicating a complicated epidemiological scenario (Kanwal *et al*., 2014). For example, in a phylogenetic analysis in 2022, the circulating strain in Pakistan (A PK C6 2017) clustered with O serotype isolates, and recombination signals were in the P2 and P3 regions of the genome. This implies that A PK C6 2017 was a potential O and A serotypes recombination (Naqvi *et al*., 2022).

In a phylogenetic study, FMDV field strains in the Sulaimani province, Iraq, belonged to the OPanAsia2 lineage and clustered with Pakistan and Iran isolates (KU365843 and KY091283) with high similarity (96.00% and 95.00%, respectively) (Sheikh *et al*., 2021). Iran shares long borders with both Pakistan and Iraq, so the presence of phylogenetically related viruses in these countries is not surprising.

Phylogenetic analysis of isolated viruses from the 2015-2016 outbreaks in Iran, revealed that the OPanAsia2 lineage is now active and dominant among all ME-SA lineages. The results of this study suggested that the OPanAsia2 vaccine had no coverage with some circulating strains in Qom, Alborz, and Zahedan and the necessity of OPanAsia2 replacement with a new vaccine. Furthermore, all isolated samples in this study have a high level of similarity to previously reported sequences from the neighboring countries. This research indicates the need for constant genetic and antigenic evaluation of clinical isolates to ascertain matching of vaccine strain with circulating variants and the need to restrict uncontrolled animal movements from the neighbor countries.

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Conflict of interest

The authors declare that they have no conflict of interest.

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