SHORT COMMUNICATION

Veterinary Research Forum. 2021; 12 (2) 247 – 251
doi: 10.30466/vrf.2019.101755.2424
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Molecular characterization and phylogenetic analysis of foot and mouth disease virus isolates in Sulaimani province, Iraq

Mohammed Baba Sheikh*, Peshnyar Atta Rashid, Zhino Raheem, Ari Salahadin Marouf, Kamaran Mohammed Amin

Department of Molecular Biology, Veterinary Laboratory Center, Sulaimani Veterinary Directorate, Sulaimani, Iraq.

Article Info

Article history:

Received: 14 January 2019
Accepted: 30 October 2019
Available online: 15 June 2021

Keywords:

Molecular genotyping
Phylogenetic analysis
Viral evolution
VP1 gene

Abstract

Foot-and-mouth disease (FMD) is endemic in Iraq. The current study can be considered as the first molecular characterization of serotype O in Iraq. The present investigation reported the determination of FMDV serotype O from local farms in Sulaimani districts in 2016 outbreaks. Samples were collected from suspected cattle. The virus was primarily detected with RT-PCR directly from mouth epithelial samples. The direct sequencing and subsequent analysis of amplified PCR products for the *VP1* gene indicated the circulation of serotype O of FMD in studied areas. Moreover, phylogenetic analysis revealed that the field isolated serotype O belonged to topotype ME-SA and lineage PanAsia II and clustered with Pakistan and Iran isolates (KU365843 and KY091283) with identity (96.00%, 95.00%) respectively. Furthermore, according to the phylogenic tree, the field isolates had different lineage with the three O/Manisa vaccine strains and Iraq/2000 strains. These findings highlighted the continuous circulation of serotype O of FMD in the region.

Introduction

Foot-and-mouth disease (FMD) is a fatal viral disease that affects cloven-hoofed animals causing a huge economic loss in domestic and wild animals worldwide.[1] FMDV belongs to Picornaviridae family of Aphthovirus genus.[2,3] Its genome is composed of a single strand of positive-sense RNA of approximately 8.4 kb in length.[4,5] FMD virus exists as seven different serologically distinct types namely, serotypes O, A,[6] C SAT 1, SAT2, SAT3 , Asia1,[7] with no cross immune protection, Infection with anyone serotype does not confer immunity against another.[8] Within serotypes, many strains can be identified by biochemical and immunological tests.[9] The disease plays an important role in global trade and is one of a priority diseases among the list A diseases published by Office International des Epizootics.[10] FMDV comprises four capsid proteins VP1, VP2, VP3, and VP4, VP1-VP3 genes encode proteins exposed on the capsid surface.[11] The VP1 contains the major antigenic determinants for infection, resulting in seven distinct genetic lineages that correlate with serotype.[12] A major highly variable antigenic site in the FMDV is located at the exposed G-H loop, comprising amino acids 134 - 160 of the capsid protein VP1.[13] The VP1 plays a significant role in cell infection and is also a primary target for conservative host responses mediated via humeral immunity.[14] The first case of FMD serotype O in Iraq was officially recorded in 1957. Other serotypes A, SAT 1 and Asia1 were recorded in 1952, 1962, and 1975, respectively. In 2007, the serotype O PanAsia-2, was detected in the Middle East as a new serotype. This new strain was probably originated from a strain circulating in India in 2001. It subsequently became pandemic in other countries such as Iran, Pakistan, Jordan, Turkey, the Palestinian Autonomous Territories, UAE, Kuwait, Bahrain, KSA, probably Lebanon and Egypt.[15,16] Previous articles about genetic characteristic of FMD type A and type Asia1 was performed in Iraq.[5,7]

In this study, FMDV serotype O was identified in cattle in Sulaimani province in 2016. This is the first study analyzing the genetic characteristics of FMDV serotype O viruses in Iraq. The phylogenetic analysis, focusing on full length *VP1* gene, provided information to identify the closely-related viruses to better understand the epidemiology of the virus in the area. Another purpose was to recommend genetically matching vaccine strains with the circulating strain for controlling programs of FMD in Iraq.

***Correspondence:**

Mohammed Baba Sheikh. DVM
Department of Molecular Biology. Veterinary Laboratory Center, Sulaimani Veterinary Directorate, Sulaimani, Iraq
E-mail: moosheik@iupiu.edu

Materials and Methods

Case history. Suspected FMD infections were reported in two herds of cattle in Jemalk and Dere village, Zharawa district of Sulaimani province (Iraq) in 2016. The district is located in Iran-Iraq border. Several symptoms were observed including anorexia, depression, frothy salivation, crusty lesion on external surface of gum and lameness. The herd consisted of 19 cattle in Dere and 38 cattle in Jemalk, cattle ages ranged between 1 - 3 years. The morbidity and mortality rates in both herds were about 100% and 15.00%, respectively. The suspected preliminary diagnosis was FMD based on clinical signs.

Samples preparation. Two epithelial tissue samples from mouth lesions were collected from a suspected FMD cattle herd. Samples were collected by Zharawa Veterinary Department. The samples were then transported on ice to the molecular diagnostic laboratory of Sulaimani Veterinary Directorate.

Extraction of RNA. Total RNA was extracted directly from mouth epithelial tissues using RNA extraction tissue kit (GeNet Bio, Daejeon, South Korea) according to manufacturer's protocol.

Oligonucleotides primer. In this study different primers sets were used, First, the universal primer set (1F: GCCTGGTC TTTCCAGGTCT /1R: CCAGTCCCCTTCTCAGATC) was designed for diagnosis of all FMD virus serotypes that amplify 328 bp of 5UTR.[17] For identification and sequencing of full length *VP1* gene of FMD serotype O these primers were used as recommended by Food and Agriculture Organization (FAO): Forward primers O1C244F(5'GCAGCAAAACACATG TCAAACACCTT- 3') and reverse primer EUR2B-52R (5'-GACATGTCCTCCTGCATC TGG TTGAT-3).[18] The primers were synthesized by (Macrogen Co., Seoul, South Korea).

Complementary DNA (cDNA) and PCR. Complete sequence of *VP1* gene was amplified by using one-step RT-PCR Premix (2X) (GeNet Bio). The reactions were carried out in 0.20 mL PCR tube based on the following specifications: 10.00 µL RT-PCR premix, 5.00 µL RNA, 1.00 µL forward (10.00 pmol), 1.00 µL reverse primers (10.00 pmol), and 3.00 µL molecular grade H2O to make up a final volume of 20.00 µL. The conventional PCR machine (Hercuvan, Carlsbad, USA) was programmed as follows: cDNA synthesis 50.00 ˚C for 30 min, initial denaturation at 95.00 ˚C for 10 min followed by 40 cycles of 95.00 ˚C for 45 sec annealing at 58.00 ˚C for 45 sec, and extension at 72.00 ˚C for 1 min and a final extension at 72.00 ˚C for 10 min. PCR was programmed for primer set (1F/1R) as follows: Denaturation 30 sec, annealing 30 sec and extension 30 sec. The PCR products were analyzed by loading 7.00 µL on standard 1.00% (w/v) agarose gel (Gendirex, Daejeon, South Korea) in 1X Tris/Borate/EDTA (TBE) buffer. The gel was stained with 4.00 µL safe dye (EURx, Banino, Poland).Electrophoresis was done on 100 v for 1 hr. After the run, the gel was removed and visualized under a UV trans-illuminator (Uvitec, Cambridge, UK).

Phylogenetic sequences analysis. Phylogenetic trees were constructed based on the complete *VP1* gene of 50 strain of FMDV serotype O. The field strains and vaccines strains of (O Manisa) were obtained from the GenBank® database. The sequence homology and multiple sequences alignment at the nucleotide and amino acid level was performed by the CLUSTALW program.[19] The phylogenetic tree, was constructed by MEGA (version 7.0; Biodesign Institute, Tempe, USA) employing the neighbor-joining (NJ) method.[20]

Results

RT-PCR and serotype detection. The suspected cattle samples were positive for FMD virus based on the agarose gel-electrophoresis. The result demonstrated the expected amplicon size of 328 bp. FMDV serotypes O were then detected by amplification of 1165 bp using FMD serotype O specific primers. The results were confirmed by sequencing of PCR product and the sequences were submitted to NCBI GenBank® and they got accession number KY412559 for ZH/SUL1/2016 strain in Jemalk village herd and accession number KY412560 for ZH/ SUL2/2016 strain in Dere village herd.

Sequences analysis and phylogenetic tree. Phylogenetic analysis was constructed based on the 55 nucleotide sequences of *VP1* gene of the field isolates and others presence sequences of serotypes O in the GenBank® database (Fig. 1). The results revealed that the field virus isolates, Zh/sul/2016 and Zh/sul2/2016 were belonged to topotype of Middle East-South Asian (ME-SA) and lineage (PanAsia-II), and clustered with KU365843/Pak/O/RYK and KY091283/IRN/18/2010 strains (Table 1). A comparative analysis of the both Sulaimani strains showed 99.00% nucleotide homology with 100% amino acid homology. The nearest identities with Sulaimani strains were KU365843/Pak/O/RYK and KY091283/IRN/18/2010 with 96.06%% and 95.31%, respectively.

Genetic analysis of Sulaimani virus with vaccine strains that used in Iraq and the sequence data available in GeneBank® showed 95.84%, 96.12%, 95.52% homology with O1/Manisa/87, O1/Manisa, and Manisa/TRK/69, respectively. However, critical amino acid substitutions determined at the VP1 GH loop positions 134 - 160 included D138E, G139S, T140R, V141A, A144T and A158T which are responsible for antigenic heterogeneity. Sulaimani isolates were also showed amino acid substitute H195Q and Q198 at critical region of carboxylic terminus, 194-201 amino acid (Fig. 2).

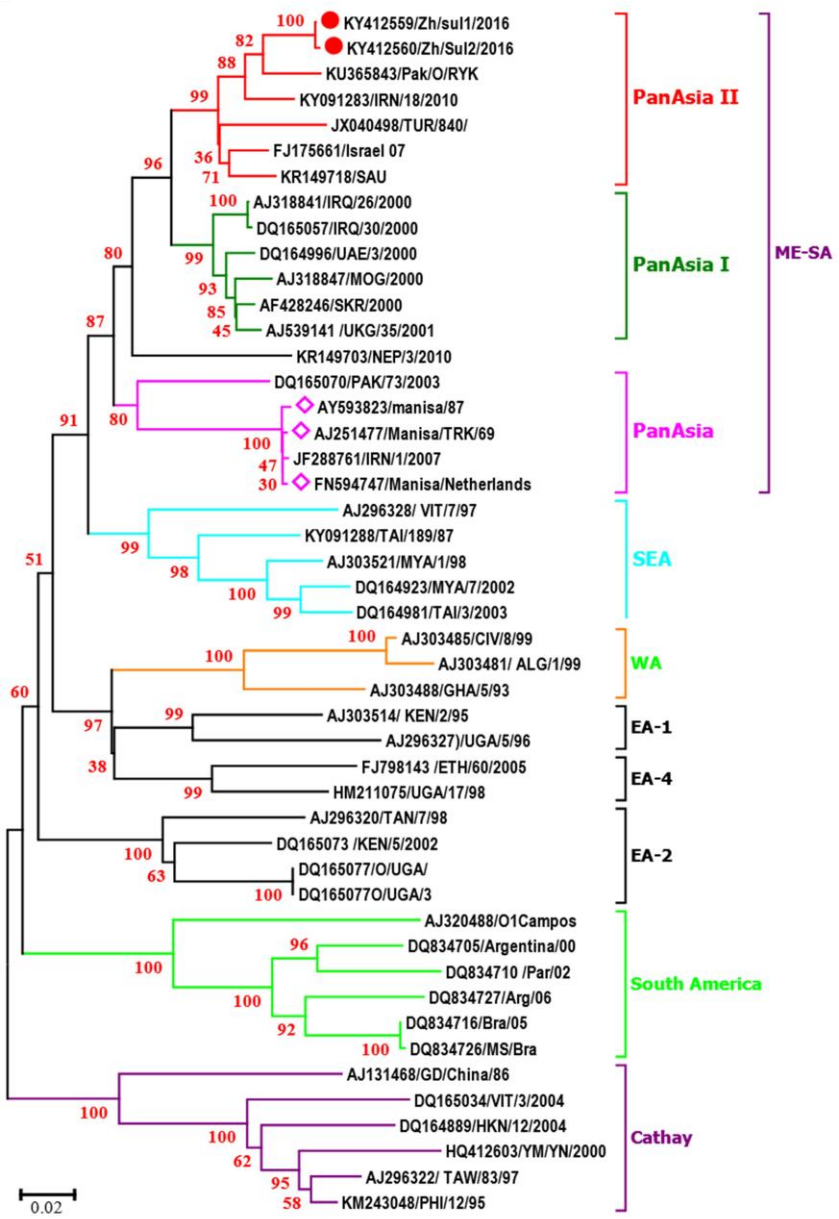

Fig. 1. Neighbor joining (NJ) phylogenetic tree generated using the nucleotide sequences for the complete VP1 coding regions of serotype O FMDVs. The red circles show Sulaimani field strains and purple rhombus – show O Manisa vaccine strains.

Table 1. Similarity between FMD Type O Zh/Sul/2016 VP1 and different topotype related isolates obtained from sequence comparison.

Accession No.	strain	Location	DNA sequence identity	a.a sequence identity	Topotype	lineage
AY593823	O1Manisa/87	Turkey	87.64	95.84	ME-SA	PanAsia
AJ251477	O1Manisa/69	Turkey	87.79	95.52	ME-SA	PanAsia
FN594747	O1Manisa	Netherlands	87.79	96.12	ME-SA	PanAsia
KU365843	Pak/O/RYK	Pakistan	96.06	97.16	ME-SA	PanAsia -II
KY091283	IRN/18/2010	Iran	95.31	96.24	ME-SA	PanAsia -II
DQ165057	O/IRQ/30/2000	Iraq	92.64	97.18	ME-SA	PanAsia -I
KM921876	/UAE/4/2008	UAE	88.52	94.37	ME-SA	PanAsia -I
KY091288	/TAI/189/87	Thailand	87.17	93.40	SEA	-
FJ798143	/ETH/60	Ethiopia	83.10	93.90	EA-4	-
KM243048	/O/PHI/12/95	Philippines	82.16	88.68	Cathay	-
DQ834726	O/MS(4)/Bra	Brazil	80.75	86.79	South America	-
AJ303481	ALG/1/99	Algeria	80.69	85.38	WA	-
AJ296327	UGA/5/96	Uganda	82.92	91.08	EA-2	-

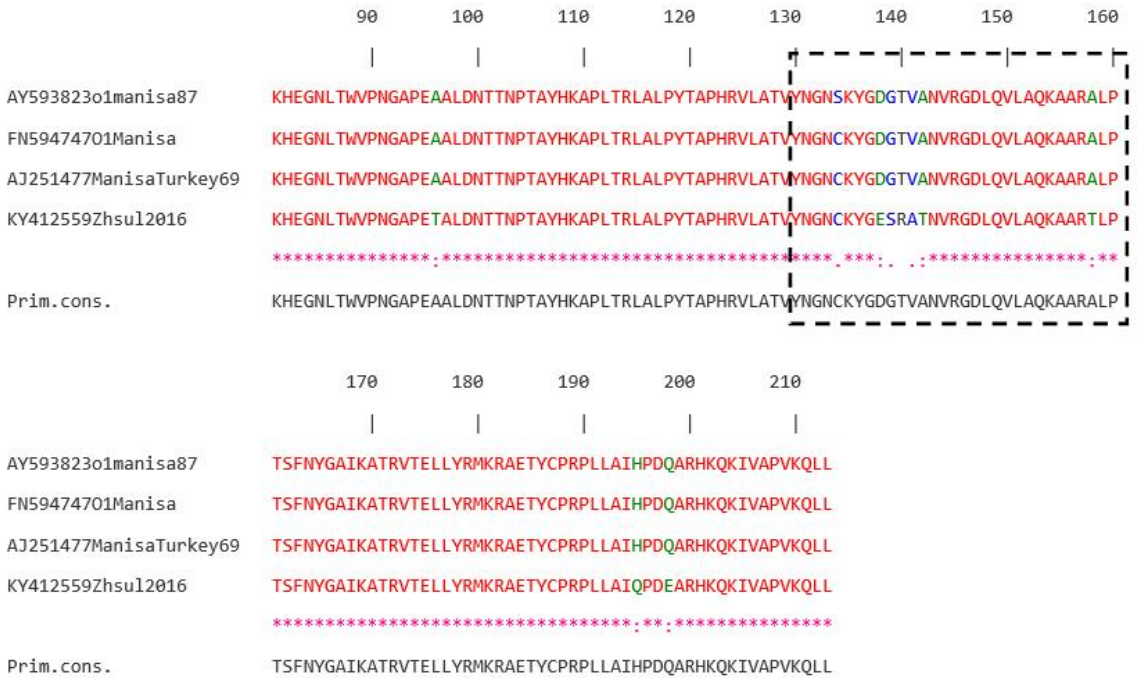

Fig. 2. Fields isolate sequences alignment with three vaccines reference. Multiple sequences alignment of the amino acid of residue at G-H loop (133-160) of VP1 region and also amino acid substitute at C terminus region position (194-2011): Fields isolates FMD serotype O with currently available vaccine strain (Manisa trk/69), Manisa trk/87 and O1Manisa/Netherlands. G-H loop showed in box.

Discussion

The FMD is the most damaging viral disease affecting cattle farming in Iraq. Recent articles recorded that serotype A and Asia1 still persist and circulating in Iraq.[5,7] This study reported the first molecular characterization of VP1 sequence data for a local strain of FMDV serotype O isolated from cattle in Iraq. RT-PCR was performed by using specific primers for FMDV serotype O to amplify the VP1 coding region of FMDV. FMD serotype O in the present study had only 92.64% identity with last Iraqi strain from 2000 (DQ165057/ IRQ/30/2000) and they clustered in different lineage in the phylogenetic tree. This indicated that at least more than one subtypes of serotype O were present in the country.

The FMDV VP1 contains major immunogenic epitopes, therefore sequence based on method of vaccine selection may be the useful tool for vaccine strain prediction models. Multiple substitutions were found in the G–H loop of VP1 according to a comparison of the amino acid sequences of VP1 region. These variations may lead to mismatching with current vaccine strains (O1 Manisa) that used for vaccination in Iraq. In FMD type A, changes in G–H loop region has been reported to be associated with changes in the antigenicity of the virus.[11,20] Million doses of vaccine are used in Iraq annually in a six-monthly vaccination strategy for controlling of FMD. However, no reports detailing the efficacy of the vaccines were currently available in Iraq. The differences between vaccines and field virus sequences may lead to insufficient immune response in the hosts. According to this study, Manisa/turkey/69 vaccine strain was better than other two vaccine strains Manisa trk/87 and O1Manisa/ Netherlands for matching with Sulaimani field strain. These findings showed important epidemiological value in distinguishing different strains of serotype O that are circulating in the region. Further antigenic, pathogenic and molecular characterizations of these isolates should be used for developing suitable prevention and control strategies for this emerging bovine pathogen. Consequently, the authorities responsible for importing and/or production of suitable vaccines should avoid import of incomparable vaccine serotypes because they may waste money more outbreaks may ensue.

In summary, the genetic characteristics of FMDV serotype O revealed variations in nucleotide sequences, however, genetic analysis indicated that the field isolated viruses were clustered with different vaccine strain and previous strain in Iraq. Thus, further molecular analyses coupled with protection potential of the existing vaccines against the isolates should be performed.

Acknowledgments

We would like to express our gratitude to the Sulaimani Veterinary Directorate for providing laboratory space for this research, as well as the Zharawa Veterinary Department for providing field samples.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

1. Kandeil A, El-Shesheny R, Kayali G, et al. Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch Virol 2013;158(3):619-627.
2. Xin A, Li L, Zhu M, et al. Whole genome sequencing of a candidate strain for FMDV vaccine: Genomic structure and genetic variation. Molecular Pathogens 2011 2(1). doi: 10.5376/mp.2011.02.0001.
3. Samuel AR, Knowles NJ. Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). J Gen Virol 2001; 82(pt3):609-621.
4. Nishi T, Ulziibat G, Khanui B, et al. Genome sequence of foot-and-mouth disease virus of serotype O lineage Ind-2001d isolated from cattle in Mongolia in 2015. Genome Announc 2017; 5(45): e01244-17. doi:10.1128/genomeA.01244-17.
5. Rashid PMA, Marouf AS, Raheem ZH, et al. Phylogenic analysis and molecular characterization of Slemani/Kurdistan/2013 foot and mouth disease virus shows circulation of new genotype in Iraq. Journal of Zankoy Sulaimani- Part A 2014; 16:3. doi:10.17656/ jzs.10298.
6. Kotecha A, Perez-Martin E, Harvey Y, et al. Chimeric O1K foot-and-mouth disease virus with SAT2 outer capsid as an FMD vaccine candidate. Sci Rep 2018; 8(1) doi:10.1038/s41598-018-31856-x.
7. Sheikh MOB, Rashid PMA, Marouf AS, et al. Phylogenic analysis of serotype Asia1 foot-and-mouth disease virus from Sulaimani/Iraq using VP1 protein: heterogeneity with vaccine strain As1/Shamir/89. Iran J Vet Res 2017;18(3):212-215.
8. Parida S. Vaccination against foot-and-mouth disease virus: strategies and effectiveness. Expert Rev Vaccines 2009;8(3):347-365.
9. Ganji VK, Kontham S, Pottabathula M. Understanding the molecular relationship between foot-and-mouth disease virus serotype O of Indian vaccine strain with strains across the world by phylogenetic analysis. Int J Curr Microbiol App Sci 2018;7(5):99-105.
10. King D, Di Nardo A, Henstock M OIE/FAO Foot-and-Mouth Disease Reference Laboratory Network Annual Report 2018: 61.
11. Mahapatra M, Statham B, Li Y, et al. Emergence of antigenic variants within serotype A FMDV in the Middle East with antigenically critical amino acid substitutions. Vaccine. 2016; 34(27):3199-3206.
12. Ahn I, Bae SE, Son HS. Comparative study of codon substitution patterns in foot-and-mouth disease virus (serotype O). Exp Mol Med 2011;43(10):587-595.
13. Logan D, Abu-Ghazaleh R, Blakemore W, et al. Structure of a major immunogenic site on foot-and-mouth disease virus. Nature 1993;362(6420):566-568.
14. Li P, Lu Z, Bao H, et al. *In-vitro* and *in-vivo* phenotype of type Asia 1 foot-and-mouth disease viruses utilizing two non-RGD receptor recognition sites. BMC Microbiol 2011;11:154.doi:10.1186/1471-2180-11-154.
15. Soleimanjahi H, Sedeh FM, Jalilian AR, et al. Sequence analysis of the foot and mouth disease virus type O/IRN/2007 *VP1* gene from Iranian isolate. Folia Biol (Praha) 2013;59(2):93-98.
16. Brückner G, Saraiva-Vieira VE. OIE strategy for the control and eradication of foot and mouth disease at regional and global levels. Conf. OIE 2010, 187-198.
17. Fowler VL, Bankowski BM, Armson B, et al. Recovery of viral RNA and infectious foot-and-mouth disease virus from positive lateral-flow devices. PloS One 2014; 9(10):e109322. doi:10.1371/journal.pone.0109322.
18. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22(22):4673-4680.
19. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16(2):111-120.
20. Lloyd-Jones K, Mahapatra M, Upadhyaya S, et al. Genetic and antigenic characterization of serotype O FMD viruses from East Africa for the selection of suitable vaccine strain. Vaccine 2017; 35(49 Pt B): 6842-6849.