

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Genetic Polymorphisms of Foot-and-Mouth Disease Virus

Khammadow Nail Ildarovich

Abstract

The aim of the work is to search for loci of the genome of various types of foot-and-mouth disease virus (FMDV), characterized by the lowest variability, for use as genetic markers in the polymerase chain reaction (PCR) of virus identification. The nucleotide sequences of the genomes of FMDV of types A, Asia-1, C, O, and SAT (1, 2, and 3) were analyzed. When aligning the genomes of isolates of each type of virus, potentially conservative sites were identified. Comparing these loci, different types of the virus have one, the most conserved locus. Subsequent basic local alignment search tool (BLAST) analysis established the correspondence of the conservative locus to the FMDV genome, and primers and a probe were developed to amplify this locus.

Keywords: foot-and-mouth disease virus, type, strain, genome polymorphism, PCR

1. Introduction

The foot-and-mouth disease virus (FMDV) is an RNA virus belonging to the genus *Aphthovirus*, family *Picornaviridae*. The FMDV includes serotypes: A, Asia-1, C, O, and SAT (1, 2, and 3) [1]. The FMDV infection causes ulcerations (vesicles) on the mucous membranes of the tongue, mouth, nares, hooves, and udder. FMDV is highly contagious. One sick animal can spread the infection rapidly to susceptible animals. The virus is released during the incubation period, in exhaled air, milk, urine, feces, sperm, and saliva. And when there are clinical signs of the disease, the virus is also isolated from the vesicular erosive lesions of the mucous membranes and skin [2].

FMDV is pathogenic to more than 100 domestic and wild animal species [3–5]. FMDV also affects humans [6].

The FMD is zoonotic. It can cause mild infection in humans. The virus infiltrates the human body through the mucous membranes. The incubation period (5–10 days) [7] of the disease is characterized by the virus reproduction in the primary focus. At the end of the incubation period, the pathogen spreads through the body (viraemia). In places where the virus enters, skin and mucous membrane blisters are formed. These blisters later transform into ulcers. The clinical manifestation of FMDV in children is more pronounced, and the clinical signs are sometimes similar to intoxication [8].

In addition to the obvious clinical picture, the detection of FMDV actively uses serological and molecular genetic diagnostic methods. For serological diagnostics, the enzyme immunoassay method is deservedly popular [9–13]. Polymerase chain

reaction and virus genome sequencing methods are used for genetic indication of FMD [14, 15]. The historical development of FMDV research covers a wide variety of techniques. In 1960, the use of cell cultures infected with FMDV was mentioned [16]. In 1966, a complement fixation test (CFT) was used to identify the FMDV [17]. The virus neutralization was employed [18] and, in 1979, the ELISA was introduced and currently in use [9, 10, 13]. The present century is also characterized by the active use of genetic analysis of FMDV nucleic acids [19].

The aim of the study is to search for universal conservative genome loci present in all types of foot-and-mouth disease virus, for use as genetic markers for PCR virus detection.

2. Materials and methods

The methodology used in this work for analysis of the genomes of the seven FMDV serotypes (Table 1) were as described for other microorganisms [20, 21]. The nucleotide sequences of the desired virus were determined by searching the National Center for Biotechnology Information (NCBI) resource databases. The strain diversity of the detected FMDV using the analyzed genetic marker was determined using the nBLAST program utility, and the nucleotide sequences of the primers and probes were designed using the Vector NTI 9.1.0 program (Invitrogen Corporation).

A positive control plasmid DNA was used for PCR amplification. PCR was performed on a C1000 amplifier with a CFX96 optical unit (BioRad). The methodology for PCR amplification is similar to that described previously [20], with the following modifications: probe for PCR, direct and reverse primers were developed in the framework of this work; the primer annealing temperature was 58.5°C; and the PCR (fluorescence) result was detected on each PCR cycle, at 58.5°C via Rox and Cy5 channels.

FMDV serotype	Isolates/strains name (NCBI coding)	GenBank ID	Source
Serotype A	Isolate <i>Zambia/90</i>	MH053307	Host “cattle,” country “Zambia: Kasama”
	Isolate 4235	JN099688	Host “cattle,” country “Iraq: Altenia, Hilla, Babil”
	Isolate <i>aphilippines iso50</i>	AY593793	Country “Philippines”
	Isolate <i>A/Pocheon/001/KOR/2010</i>	KC588943	Host “cattle,” country “Iraq: Altenia, Hilla, Babil”
	Isolate <i>MAY/23/2013</i>	KY322678	Host “cattle,” country “South Korea”
	Isolate <i>A01L</i>	KY404934	Country “Argentina”
	Isolate <i>A/NIG/3/15</i>	MG725874	Host “Bos taurus,” country “Nigeria”
Serotype Asia-1	Isolate <i>BAN/TA/Ma-167/2013</i>	MF782478	Host “cattle,” country “Bangladesh: Madhupur, Tangail”
	Isolate <i>As/SIN/PAK/L2810/2009</i>	JN006720	Host “cattle,” country “Iraq”
	Strain <i>Asia-1/Jiangsu/China/2005</i>	EF149009	Isolation source “bovine vesicular tongue tissue lysate,” country “China: Jiangsu, Wuxi”
	Isolate <i>IND 101-99</i>	DQ989310	Host “cattle,” country “India”
	Isolate <i>IND 37-02</i>	DQ989311	Host “buffalo,” country “India”

FMDV serotype	Isolates/strains name (NCBI coding)	GenBank ID	Source
Serotype C	Strain C-S8p200	FJ824812	Country “Spain”
	Isolate <i>c1noville iso56</i>	AY593804	Plum Island Animal Disease Center Virus Collection, isolated 1965, country “Switzerland”
	Isolate <i>cwald iso32</i>	AY593810	Plum Island Animal Disease Center Virus Collection, isolated 1970, country “the United Kingdom”
	Isolate <i>KEN/1/2004</i>	KM268897	Host “cattle,” country “Kenya: Koibatek, Rift Valley”
	Isolate <i>ETH/1/71</i>	MH053308	Host “unknown,” country “Ethiopia”
	Isolate <i>KEN/32/70 (K267/67)</i>	MH053309	Host “cattle,” country “Kenya: Nanyuki, Laikipia”
	Isolate <i>UGA/18/70</i>	MH053310	Host “cattle,” country “Uganda: Acholi district”
Serotype O	Isolate <i>UGA/3/2002</i>	MH053318	Host “unknown,” country “Uganda: Nakasongola district”
	Strain <i>O/YM/YN/2000</i>	HQ412603	Host “Sus scrofa,” country “China”
	Isolate <i>o11indonesia iso52</i>	AY593813	Plum Island Animal Disease Center Virus Collection, isolated 1962, country “Indonesia”
	Isolate <i>BAN/NA/Ha-156/2013</i>	KF985189	Host “cattle,” country “Bangladesh”
Serotype SAT 1	Isolate <i>SAT1/NIG/1/15</i>	MF678823	Host “Bos taurus,” country “Nigeria”
	Isolate <i>SAT1/NIG/2/15</i>	MF678824	Host “Bos taurus,” country “Nigeria”
	Isolate <i>SAT1/NIG/3/15</i>	MF678825	Host “Bos taurus,” country “Nigeria”
Serotype SAT 2	Isolate <i>EGY/9/2012</i>	JX014255	Country “Egypt: El-Suiz”
	Isolate <i>PAT/1/2012</i>	JX014256	Host “cattle,” country “Gaza Strip: Palestinian Autonomous Territories, Rafa”
	Isolate <i>EGY/3/2012</i>	KC440884	Host “cattle,” country “Egypt: Garbia Governorate”
Serotype SAT 3	Isolate <i>ZIM/P27/90(DSA-31)</i>	MH053352	Host “ <i>Syncerus caffer</i> ,” country “Zimbabwe: Dande Safari Park”
	<i>Triticum aestivum</i> chromosome 2	LS480641	Organism “ <i>Triticum aestivum</i> ”

Table 1.
Nomenclature of isolates/strains of FMDV and other organisms described in the work.

3. Results and discussion

3.1 Epidemiology

Scientific publications on the infection of humans with the FMDV began to appear as early as 1869, 1872 [22, 23]. After some time, seven different serotypes of FMDV were identified, which have a different distribution. Some serotypes have a restricted geographical distribution, for example, Asia-1, whereas others, notably serotype O, occurred in many different regions [24].

FMDV is a very dangerous disease, and the data from the Pakistan showed that mortality due to the dominant FMDV serotype “O” was from 7.74 to 21.61% [25].

Studies in Nigeria showed a high prevalence of FMDV in the wild. Thus, the incidence of cattle ranges from 39.7 to 72.8% (in various animal breeds). Also, antibodies to FMDV were found in waterbucks, elephant, wildebeests, and other animals [26]. It is important to note that the protective properties of vaccines are effective only against infection with the same subtype [24].

3.2 Genetic analysis

The following is an analysis of the variability of the nucleotide sequences of various isolates for each of the virus serotypes (the list of isolates is shown in **Table 1**). For ease of orientation in the nucleotide sequence of the genomes of the various isolates of FMDV serotype A, the designations will be indicated relative to the isolate Zambia/90 (GenBank ID MH053307). For orientation in the nucleotide sequence of the genomes of various isolates of FMDV serotype Asia-1, designations will be indicated relative to isolate BAN/TA/Ma-167/2013 (GenBank ID MF782478). For orientation in the nucleotide sequence of the genomes of various isolates of FMDV serotype C, designations will be indicated relative to strain C-S8p200 (GenBank ID FJ824812). For orientation in the nucleotide sequence of the genomes of various isolates of FMDV serotype O, designations will be indicated relative to isolate UGA/3/2002 (GenBank ID MH053318). For orientation in the nucleotide sequence of the genomes of various isolates of FMDV SAT serotypes (1, 2, and 3), designations will be indicated with respect to SAT 3 serotype, isolate ZIM/P27/90 (DSA-31) (GenBank ID MH053352). Orientation in the nucleotide sequence of the genomes of various serotypes of FMDV, when analyzing the nucleotide sequence encoding the pathogenicity factor of the virus, will be labeled with respect to serotype A, isolate 4235 (GenBank ID JN099688). Analysis of the genomes of all serotypes is aimed at identifying the universal locus (marker nucleotide sequence), which is available in all FMDV serotypes. The marker locus should have minimal variability (minimum number of nucleotide substitutions in the annealing region of primers and probes). The size of the marker locus should not exceed 200 bp (base pair).

The identification of homologies among various types of virus, within the locus encoding the pathogenicity factor “VP1,” is aimed at determining the locus, during amplification of which the maximum number of isolates (or all isolates) will be detected in all serotypes of FMDV. This nucleotide sequence is part of the nucleotide sequence encoding a viral capsid. The nucleotide sequence is located in the genome of the virus in the area from 2703 to 3353 bp. There were no significant homologies at this locus (40.5% homology), and the FMDV indication for this locus is not informative.

Further analysis was aimed at identifying homologies among the most abundant (largest number strains/isolates) serotype of the virus type, namely type O (179 strains/isolates). In the analysis, sequences were selected in which no more than two nucleotide substitutions were found in each region of the generation of oligonucleotide seeds (for different isolates). The first locus, in which it is possible to design oligonucleotide seeds, is localized in the region from 4132 to 4292 bp, while the maximum variability was observed in the strain with ID HQ412603 and isolate with ID AY593813. The second locus, in which it is possible to design oligonucleotide seeds, is localized in the region from 7810 to 7908 bp, while the maximum variability was observed in the isolate with ID KF985189; and the third locus, in which it is possible to design oligonucleotide seeds, is localized in the region from 7913 to 8043 bp; no significantly variable strains/isolates were detected in the nucleotide sequence of the locus.

Further homology was detected among the next most numerous virus strains/isolates, type A (117 strains/isolates). Homology was detected at three

loci, which, based on the results of the analysis of type O virus, were able to design oligonucleotide seeds. By analyzing the first locus, which is localized in the region from 4151 to 4310 bp, one isolate with three nucleotide substitutions was identified in the region of the generation of the reverse primer (AY593793). Analysis of the second locus located in the region from 7829 to 7926 bp revealed three variable isolates (KC588943, KY322678, and KY404934). Analysis of the third locus located in the region from 7933 to 8061 bp revealed one isolate (MG725874) with three nucleotide substitutions in the region of generation of the oligonucleotide probe.

The identification of homologies among virus isolates, type Asia-1 (59 strains/isolates), was continued at the same three loci. By analyzing the first locus located in the region from 4162 to 4321 bp, one isolate (JN006720) with three nucleotide substitutions was detected in the region of generation of the oligonucleotide probe. The second locus, located in the region from 7840 to 7937 bp, was characterized by the variability of three nucleotides in the region of generation of the oligonucleotide probe in one strain (EF149009). Analysis of the third locus located in the region from 7944 to 8072 bp revealed polymorphism in the region of generation of the oligonucleotide probe in two virus isolates (DQ989310, DQ989311).

Virus isolates, type C (23 strains/isolate), were further analyzed for homology. The first locus is localized in the region from 4100 to 4259 bp, with maximum variability observed in six virus isolates (AY593804, AY593810, KM268897, MH053308, MH053309, and MH053310). The second locus, localized in the region from 7778 to 7875 bp, with maximum variability observed in three virus isolates (AY593810, MH053308, and MH053310). And the third locus, located in the region from 7882 to 8010 bp, in the nucleotide sequence of which no significantly variable strains/isolates were detected.

The identification of homologies among virus isolates, type SAT, was carried out for a total of 74 strains/isolates for all three variants (SAT1, SAT2, and SAT3). The first locus is localized in the region from 4137 to 4296 bp, with maximum variability observed in more than 21 strain/isolates of the virus. The second locus is localized in the region from 7812 to 7909 bp, while the maximum variability was observed in 23 virus strains/isolates. And the third locus, localized in the region from 7916 to 8044 bp, with the maximum variability observed in six isolates of the virus (JX014255, JX014256, KC440884, MF678823, MF678824, and MF678825 were found to have identical nucleic substitutions, and the presented isolates had identical nucleotide sequences at the analyzed locus). An additional probe allows minimizing the effect of nucleotide substitutions on the FMDV indication (the nucleotide composition of the probe contains three substitutions characteristic of the above isolates).

Thus, the identification of homology among strains/isolates of all FMDV serotypes allowed us to determine the locus with minimal variability (in the text, this is the third locus). At this locus, oligonucleotides are complementary to the following positions in the GTA/3/2002 virus genome (GenBank ID MH053318): forward primer 7913–7934 bp, reverse primer 8026–8043 bp, and probe 7988–8024 bp.

All oligonucleotide seeds were analyzed for specificity in the nBLAST software utility. As a result of the analysis, the high specificity of the analyzed nucleotide sequences to the FMDV genome was established. In a more detailed analysis, excluding the nucleotide sequences of the FMDV genome in the search parameters, the complementarity of the forward and reverse primers was found for only one genome, *Triticum aestivum* (soft wheat), and more precisely, its second chromosome (GenBank ID LS480641.1); amplification is not possible in this case because of the large distance between the primers (527, 987, and 115 bp). The nucleotide sequences of the probes showed an identity only with the FMDV genome.

3.3 Design of oligonucleotides

In addition to specific oligonucleotide seeds, oligonucleotide seeds were designed to control the amplification reaction; in this case, cattle genes served as DNA markers, namely, the gene encoding the milk protein and the gene encoding the fat milk of cattle.

Within the above loci to designate the genome of the foot-and-mouth disease virus and DNA markers of cattle, primers and probes for PCR were designed (Table 2) with the following requirements: the same melting temperature of the primers ($\pm 0.5^{\circ}\text{C}$); minimum dimers and secondary structures; minimum GC at 3' end; for the probe, the absence of G at the 5' end (first nucleotide) and, most importantly, the minimum variability of the nucleotide sequence (maximum two) in the sequence of each oligonucleotide seed.

Result of the design of oligonucleotide seeds, both specific and for amplification control, is presented in Table 2.

Thus, the indication of all strains/isolates of all serotypes of FMDV is achieved using three modifications of the oligonucleotide probe; the first probe (P FMDV) is used to identify the main number of virus strains/isolates; the second modification of the probe (Pas FMDV) allows the detection of two isolates not detected by the previous probe viruses; serotype Asia-1, polymorphism features of nine isolates of

Detectable pathogen/marker	Primer name	Nucleotide sequence 5' \longrightarrow 3'
Foot-and-mouth disease virus	Fp FMDV	atctccgtggcaggactcgc
	Rp FMDV	tgggtgaacgccgtgtgc
	P FMDV	Rox-ttgagattccaagctacagatcactttacctgc-BHQ2
	Pas FMDV	Rox-ttcgagataccaagctacagatcgctctacctgc-BHQ2
	Psat FMDV	Rox-ttgagatccctagctacagatcactttacctgc-BHQ2
The gene encoding the milk production of cattle	Fp kappa	ttggcaggcacagtatttgaca
	Rp kappa	attactaccaacagaaaccagttgca
	P kappa	Cy5-ttgaagaatttgggcagggtgacctaaactg-RTQ3
The gene encoding the fat milk content of cattle DGAT	Fp DGAT	cctcttctcaagctgttctctac
	Rp DGAT	cctcaccagccttggcctt
	P DGAT	Cy5-acgtcaacctctggtgccgagagc-RTQ3

Table 2.
The nucleotide sequence of primers and probes for PCR.

Analyzed oligonucleotides for internal control of amplification	Fp FMDV	Rp FMDV	P FMDV	Pas FMDV	Psat FMDV
Fp kappa	—	—	—	—	—
Rp kappa	—	—	51.9%	—	51.9%
P kappa	—	—	—	—	—
Fp DGAT	—	—	—	—	—
Rp DGAT	—	—	23.2%	—	—
P DGAT	—	31.3%	—	—	—

Table 3.
Homology (%) of target oligonucleotides with test oligonucleotides to control amplification.

serotypes SAT1 and SAT2 and one isolate of serotype A, when they are indicated, the third probe is taken into account (Psat FMDV).

An analysis of the compatibility of specific and controlling PCR oligonucleotide seeds is presented in **Table 3**.

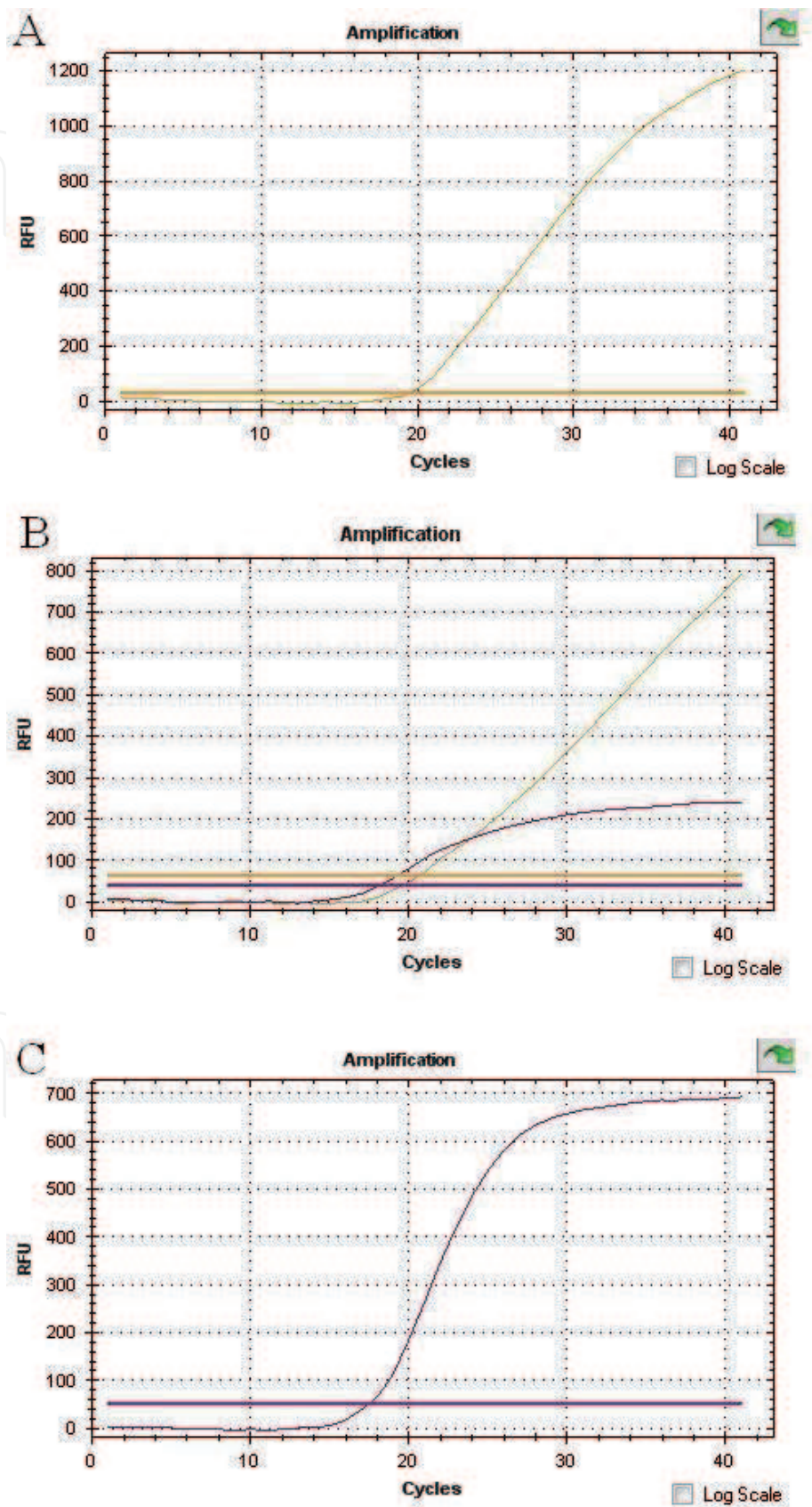


Figure 1.
Amplification of marker locus for indication of foot-and-mouth disease virus: amplification of plasmid control (A), amplification of plasmid control and cattle DNA (B), and cattle DNA amplification (C).

To control amplification, a locus was chosen within the framework of the gene encoding the milk production of cattle, since its oligonucleotide seeds are characterized by the absence of homologies with target primers to indicate FMDV, and as a result, will have minimal effect on amplification with specific primers.

The designed primers for indicating FMDV have a melting point ($58.6^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$), and the primers for amplification control have a melting point ($55.45^{\circ}\text{C} \pm 0.15^{\circ}\text{C}$), and such a temperature difference implies more favorable amplification conditions for specific primers (at an annealing temperature of 58.5°C) and minimizes the effect of oligonucleotides to control amplification on the course of the reaction.

To control the amplification result, a positive control was created. Specific marker of positive control has the following nucleotide sequence (5'-atctccgtggcag-gactcgccgtccactctggacctgacgagtaccggcgctctctttgagcccttcagggtctctttgagattccaagcta-cagatcactttacctgcgttggtgaacgccgtgtgc-3') for the indication of FMDV in plasmid DNA. The insertion of the marker sequence into the plasmid "pAL2-T" was ordered at ZAO Evrogen. To test the operability of the developed oligonucleotide seeds and plasmid control, cattle DNA and plasmid control were amplified with the primers and probes, developed above (specific for amplification control). The amplification result is shown in the **Figure 1**.

Amplification with oligonucleotide seeds for the indication of FMDV was effective both in separate PCR with positive plasmid control and in combination with cattle DNA. Amplification of the FMDV genetic markers and the control locus was performed in a single test tube. The plasmid DNA concentration was 1×10^8 DNA copies/ μl (designations in **Figure 1A** and **B**). With separate amplification with cattle DNA, cross-reactions with primers/probes for indication and for foot-and-mouth disease did not occur. Amplification with oligonucleotide seeds for detecting FMDV was indicated on the Rox channel, and internal amplification was controlled on the Cy5 channel.

4. Conclusion

An analysis of the variability of the nucleotide sequences of the genomes of the different FMDV strains/isolates within each serotype revealed a locus characterized by maximum conservatism. In the sequence of oligonucleotide seeds, a sufficient level of polymorphism in the genomes of the virus isolates was found only with respect to the PCR probe (in 12 isolates by serotypes A, Asia-1, SAT1, and SAT2), and the effect of such variability on the number of detected virus isolates allows modification of the PCR probe (Pas FMDV and Psat FMDV).

Conflict of interest

The author declares no conflict of interest.

Bioethics

This work was carried out without the use of animals.

IntechOpen

IntechOpen

Author details

Khammadov Nail Ildarovich
Federal Center for Toxicological, Radiation and Biological Safety, Kazan City,
Russian Federation

*Address all correspondence to: nikhammadov@mail.ru

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Yousef MR, Mazloum KS, Al-Nakhli HM. Serological evidence of natural exposure of camels (*Camelus dromedaries*) to foot and mouth disease virus. *Veterinary World*. 2012;5(4):197-200. DOI: 10.5455/vetworld.2012.197-200
- [2] Musser JMB. A practitioner's primer on foot-and-mouth disease. *Journal of the American Veterinary Medical Association*. 2004;224(8):1261-1268. DOI: 10.2460/javma.2004.224.1261
- [3] Bo LL, Lwin KS, Ungvanijban S, Knowles NJ, Wadsworth J, King DP, et al. Foot-and-mouth disease outbreaks due to an exotic serotype Asia-1 virus in Myanmar in 2017. *Transboundary and Emerging Diseases*. 2019;66(2):1067-1072. DOI: 10.1111/tbed.13112
- [4] Taylor S, Khan M, Zaidi S, Alvi U, Fatima Y. Central serous retinopathy and hand-foot-mouth disease: Coincidence or causation? *International Medical Case Reports Journal*. 2018;11:277-282. DOI: 10.2147/IMCRJ.S181088
- [5] Sikombe TKW, Mweene AS, Muma J, Kasanga C, Sinkala Y, Banda F, et al. Serological survey of foot-and-mouth disease virus in buffaloes (*Syncerus caffer*) in Zambia. *Veterinary Medicine International*. 2015:264528. DOI: 10.1155/2015/264528
- [6] Miller RS, Sweeney SJ, Sloodmaker C, et al. Shwiff cross-species transmission potential between wild pigs, livestock, poultry, wildlife, and humans: Implications for disease risk management in North America. *Scientific Report*. 2017;7:7821. DOI: 10.1038/s41598-017-07336-z
- [7] Yadav S, Stenfeldt C, Branam MA, Moreno-Torres KI, Holmstrom LK, Delgado AH, et al. Parameterization of the durations of phases of foot-and-mouth disease in cattle. *Frontiers in Veterinary Science*. 2019;6:263. DOI: 10.3389/fvets.2019.00263
- [8] Lizasoain A, Piegas S, Victoria M, Da Silva EE, Colina R. Hand-foot-and-mouth disease in Uruguay: Coxsackievirus A6 identified as causative of an outbreak in a rural childcare center. *Journal of Medical Virology*. 2020;92(2):167-173. DOI: 10.1002/jmv.25590
- [9] Crowther JR, Abu Elzein EME. Application of the enzyme linked immunosorbent assay to the detection and identification of foot-and-mouth disease viruses. *Journal of Hygiene*. 1979;83(3):513-519. DOI: 10.1017/S002217240002636X
- [10] Abu Elzein EME, Crowther JR. The specific detection of foot-and-mouth disease virus whole particle antigen (140S) by enzyme labelled immunosorbent assay. *Journal of Hygiene*. 1979;83(1):127-134. DOI: 10.1017/S0022172400025894
- [11] Abu Elzein EME, Crowther JR. Serological comparison of a type sat 2 foot-and-mouth disease virus isolate from Sudan with other type SAT 2 strains. *Bulletin of Animal Health and Production in Africa [Bulletin des sante et production animales en Afrique]*. 1979;27(4):245-248
- [12] Crowther JR, Abu Elzein EME. Detection of antibodies against foot-and-mouth disease virus using purified *Staphylococcus A* protein conjugated with alkaline phosphatase. *Journal of Immunological Methods*. 1980;34(3):261-267. DOI: 10.1016/0022-1759(80)90053-8
- [13] Abu Elzein EME, Crowther JR. Differentiation of foot-and-mouth disease virus strains using a competition

enzyme-linked emmunosorbent assay. *Journal of Virological Methods*. 1982;3(6):355-365. DOI: 10.1016/0166-0934(82)90040-4

[14] Howson ELA, Orton RJ, Mioulet V, Lemb T, King DP, Fowler VL. Goprime: Development of an in silico framework to predict the performance of real-time PCR primers and probes using foot-and-mouth disease virus as a model. *Pathogens*. 2020;9(4):303. DOI: 10.3390/pathogens9040303

[15] Hicks HM, Wadsworth J, Azhar M, Afzal M, Manzoor S, Abubakar M, et al. Genome sequences of foot-and-mouth disease virus O/ME-SA/Ind-2001e strains isolated in Pakistan. *Microbiology Resource*. 2020;9(18):e00165-20. DOI: 10.1128/MRA.00165-20

[16] Polatnick J, Bachrach HL. Metabolic studies of bovine kidney cultures infected with foot-and-mouth disease virus. *Virology*. 1960;12(3):450-462. DOI: 10.1016/0042-6822(60)90166-5

[17] Cowan KM, Graves JH. A third antigenic component associated with foot-and-mouth disease infection. *Virology*. 1966;30(3):528-540. DOI: 10.1016/0042-6822(66)90128-0

[18] Hardy MM, Moore DM. Neutralization of foot-and-mouth disease virus. II. Further parameters related to the sensitization of the 140S virion by antibody. *Journal of General Virology*. 1982;62(2):287-295. DOI: 10.1099/0022-1317-62-2-287

[19] Klein J, Hussain M, Ahmad M, Normann P, Afzal M, Alexandersen S. Genetic characterisation of the recent foot-and-mouth disease virus subtype A/IRN/2005. *Virology Journal*. 2007;4:122. DOI: 10.1186/1743-422X-4-122

[20] Khammadoov NI, Aleksandrova NM, Khammadoova AV, Shuralev EA.

Evaluation of the effectiveness of genetic markers of mycobacteria for assessing the disinfection quality by viability real time PCR. *BioNanoScience*. 2019;9(4):918-927. DOI: 10.1007/s12668-019-00654-8

[21] Khammadoova AV, Shuralev EA, Khammadoov NI, Oumarou BM, Faizov TK, Mukminov MN. Design of primers for identification of honey bee viruses in multiplex-PCR. *Astra Salvensis*. 2017;1(5):481-489

[22] Foot and mouth disease in relation to the meat and milk supply. *The Lancet*. 1869;94(2408):589-591. DOI: 10.1016/S0140-6736(02)66937-5

[23] Briscoe WT. A case of foot-and-mouth disease in the human subject. *British Medical Journal*. 1872;2(617):464. DOI: 10.1136/bmj.2.617.464

[24] Jamal SM, Belsham GJ. Foot-and-mouth disease: Past, present and future. *Veterinary Research*. 2013;44(1):116. DOI: 10.1186/1297-9716-44-116

[25] Abubakar M, Khan E, Arshed MJ, Hussain M, Ali Q, Afzal M. Mortality rate in association with foot and mouth disease outbreaks in cattle and buffaloes, Pakistan. *ASM Science Journal*. 2013;7(2):139-143 ISSN: 18236782

[26] Atuman YJ, Kudi CA, Abdu PA, Okubanjo OO, Abubakar A, Wungak Y, et al. Seroprevalence of foot and mouth disease virus infection in some wildlife and cattle in Bauchi State, Nigeria. *Veterinary Medicine International*. 2020;3642793. DOI: 10.1155/2020/3642793