Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v25e-76304E

e-ISSN 1809-6891
Veterinary Medicine | Research article

Comparison of seven different RT-qPCR for diagnostic of Foot-and-Mouth disease virus

Comparação de sete diferentes RT-qPCR para detecção do virus da febre aftosa

Antonio Augusto Fonseca Junior*[1], Mateus Laguardia-Nascimento[1], Amanda Petrina Scotá Ferreira[1], René Ribeiro da Silva[2], Anselmo Vasconcelos Rivetti Júnior[1], Marcelo Fernandes Camargos[1]

[1]Laboratório Federal de Defesa Agropecuária de Minas Gerais (LFDA/MG), Pedro Leopoldo, Minas Gerais, Brazil
[2]Laboratório Federal de Defesa Agropecuária do Pará, Belém, Pará, Brazil
*corresponding author: antonio.fonseca@agro.gov.br

Abstract: The prompt and accurate diagnosis of foot-and-mouth disease outbreaks is crucial to curb the swift transmission of its causative agent, the foot-and-mouth disease virus (FMDV), which poses a substantial threat to the nation's livestock. This study aimed to perform a comparative analysis of various RT-qPCR methods for the detection of FMDV. The methods evaluated for the 3D region demonstrated similar sensitivity and specificity. Nevertheless, noticeable distinctions emerged in samples with low RNA concentration. While these methods can be interchangeably utilized with consistent outcomes when handling a large number of samples sent to the laboratory to pinpoint an outbreak, minor variations may assume significance when working with a limited quantity of samples containing low FMDV RNA copies.

Keywords: RT-qPCR; foot-and-mouth disease; diagnosis; repeatability

Resumo: O diagnóstico rápido e preciso de surtos de febre aftosa é crucial para conter a rápida transmissão de seu agente causador, o vírus da febre aftosa (FMDV), que representa uma ameaça significativa ao gado do país. Este estudo teve como objetivo realizar uma análise comparativa de vários métodos de RT-qPCR para a detecção do FMDV. Os métodos avaliados para a região 3D demonstraram sensibilidade e especificidade semelhantes. No entanto, foram observadas diferenças notáveis em amostras com baixa concentração de RNA. Embora esses métodos possam ser utilizados de forma intercambiável com resultados consistentes ao lidar com um grande número de amostras enviadas ao laboratório para identificar um surto, variações sutis podem assumir importância ao trabalhar com uma quantidade limitada de amostras contendo baixas cópias de RNA do FMDV.

Palavras-chave: RT-qPCR; febre aftosa; diagnóstico; repetibilidade

Received: May 31, 2023. Accepted: November 14, 2023. Published: January 08, 2024.

1. Introduction

Outbreaks of foot-and-mouth disease demand swift and precise diagnosis to thwart the rapid dissemination of its causal agent, the foot-and-mouth disease virus (FMDV), capable of causing substantial harm to a nation's livestock [1]. FMDV is classified under the genus Aphthovirus, family Picornaviridae, and is a positive-sense single-stranded RNA virus[2]. Clinical signs of the disease include fever, restlessness, salivation, difficulty chewing and swallowing food, tremors, decreased milk production, foot lesions, and lameness. However, the most distinctive feature of the disease is the vesicles that can appear in the mouth, on the teats, and on the digits of the animals [1].

The widespread dissemination of FMDV within herds is a notable concern, amplified by the heightened global trade and movement of animals in recent years. Vigilance is crucial, given that the virus can be transmitted through direct contact among animals or via animal products. A case in point is the 2005 outbreak in Brazil, which was probably triggered by the illicit transit of animals[3], while the 2001 outbreak in the United Kingdom occurred due to feeding pigs with contaminated food scraps [4].

The first step towards an accurate diagnosis is adequate and rapid detection in the field, involving the collection of samples for laboratory testing. Rapid and reliable detection of FMDV is crucial in combating the disease. The methods employed must be sensitive enough to detect minute amounts of the virus and specific enough to avoid misdiagnosis, which can lead to delays and substantial losses. The impact of a positive FMDV diagnosis on a country's livestock can persist for months or even years until a virus-free and vaccination-free status is achieved [5].

The primary methods for detecting FMDV include serological, viral isolation, and molecular techniques. Molecular methods play a critical role in laboratory diagnosis, particularly in detecting viral RNA. Specimens used for testing typically include nasal, oral, or lesion swabs, epithelial tissue, vesicular fluid, or oral fluid [6]. The 3D region is the primary target for oligonucleotides in molecular methods, although regions such as IRES and 5' UTR are also utilized [7-9]. These three regions are highly conserved among the assortments, theoretically allowing the detection of all of them. Nonetheless, there are situations in which particular isolates from specific regions may go undetected, emphasizing the necessity of utilizing more than one PCR. VP1 is another target for RT-PCR, primarily used for serotype differentiation [10].

Thorough development and evaluation of diagnostic methods are essential, particularly for a significant disease like FMD. Technique validation is crucial, including testing for factors such as RNA extraction, matrix effect, measurement uncertainty, and precision [11]. Oligonucleotides may require regular updates to ensure accurate detection of different virus strains, as FMDV's RNA genome exhibits unique evolutionary characteristics [12]. Endemic regions experience the circulation of various serotypes and topotypes of the foot-and-mouth disease virus (FMDV), leading to considerable local consequences. Moreover, introductions into new areas are a source of significant concern. Certainly, in recent years, there have been numerous outbreaks of FMDV associated with topotypes that have transcended borders, manifesting in varied regions worldwide. In both scenarios, swift and precise diagnosis, including the determination

of the serotype and topotype responsible for these outbreaks, is vital for implementing the most efficient and suitable measures to contain the disease's spread [13]. Interlaboratory testing is common in countries with decentralized diagnostic structures and among countries that undergo regular proficiency testing to evaluate their assays [10].

The objective of this study was to conduct a comparative analysis of different RT-qPCR methods to detect FMDV. The methods were compared based on amplification efficiency, detection limit, repeatability, and diagnostic sensitivity.

2. Material and methods

2.1 RT-qPCR

The tested techniques and reagent concentrations are listed in Table 1. All tests were performed using the QuantiNova Probe RT-PCR master mix (Qiagen, Germany). The entire experiment was conducted in the CFX96 thermocycler (BioRad, United States). The RT-qPCR FMDV.3D.183 was modified for Taqman chemistry, utilizing the probe described in Table 1. We opted for this modification in an attempt to adapt a commonly used qPCR chemistry method. The RT-qPCR FMDV.3D.99 [14] was also modified by incorporating new oligos to ensure the detection of viral strains with misalignment when compared to the GenBank sequences. All RT-qPCRs were compared to FMDV.3D.107. a technique suggested by the World Organization for Animal Health.

Table 1 Oligonucleotides and PCR protocols used in this study.

RT-qPCR	Oligonucleotide	Sequence	PCR Mix	Reference
FMDV.3D.107	FMDV.3D.107.F	ACTGGGTTTTACAAACCTGTGA	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.4 µM, Probe 0.2 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(7)
	FMDV.3D.107.R	GCGAGTCCTGCCACGGA		
	FMDV.3D.107.S	FAM-TCCTT TGCAC GCCGT GGGAC-BHQ1		
FMDV.3D.99	FMDV.3D.99.F1	ACTGGGTTTTACAAACCTGTGATG	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.4 µM, Probe 0.2 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(24)
	FMDV.3D.99.F2	CTGGGTTTTATAAACCTGTGATGGC		
	FMDV.3D.99.R1	CCACGGAGATCAACTTCTCCT		
	FMDV.3D.99.R2	TGCCACAGAGATCAACTTCTCC		
	FMDV.3D.99.R3	CCACGGAAATCAACTTCTCCTG		
	FMDV.3D.99.S	FAM-TCTCCTTTGCAC-GCCGTGG-BHQ1		
FMDV.3D.130	FMDV.3D.130.F	GGACCATACAGGAGAAGTTGA	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.4 µM, Probe 0.2 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(25)
	FMDV.3D.130.R	CGCAGGTAAAGTGATCTGTAGC		
	FMDV.3D.130.S	FAM-CTCCGTGGCAG-GACTCGCAGT-BHQ1		

FMDV.3D.183	FMDV.3D.183.F	GAC AAA GGT TTT GTT CTT GGT CA	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.6 µM, Probe 0.3 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(26)
	FMDV.3D.183.R	TGCGAGTCCTGCCACGGA		
	FMDV.3D.183.S	FAM-ATC CTC TCC TTT GCA CGC CGT GGG ACC AT-BHQ1		
FMDV.IRES.145	FMDV.IRES.145.F	TAA CAW GGA CCC RCS GGG CC	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.4 µM, Probe 0.2 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(27)
	FMDV.IRES.145.R	TGA AGG GCA TCC TTA GCC TG		
	FMDV.IRES.145.S	FAM - CAT GTG TGC AAY CCC AGC ACR G - BHQ		
FMDV.3D.88	FMDV.3D.88.F	ACTGGGTTTTAWAAACCTGTGATG	12.5 µL QuantiNova RT-qPCR (Qiagen, Germany), Primers 0.4 µM, Probe 0.2 µM, 3 µL DNA sample, water q.s. 25 µL (1) 95°C 5 minutes, (45) 95°C 10 seconds, 60 °C 60 seconds	(17)
	FMDV.3D.88.R	TCAACTTCTCCTGKATGGTCCCA		
	FMDV.3D.88.S	FAM-ATCCTCTCCTTTG-CACGC-Iowa Black		

2.2 RNA Extraction

The RNA from all samples tested in this study was extracted using Trizol (Thermo Fisher Scientific, United States). 200 µL of viral suspension or 50 mg of tissue was added to 1 mL of Trizol. Subsequently, 200 µL of chloroform was added to the solution. After homogenization, centrifugation was performed at 12,000 g for 15 minutes. The supernatant was collected and mixed with 500 µL of isopropanol. It was then centrifuged at 12,000 g for 10 minutes. The supernatant was discarded, and 1,000 µL of ethanol was added. Centrifugation was carried out at 7,500 g for 5 minutes. The supernatant was discarded, and the precipitate was resuspended in 100 µL of distilled water.

2.3 Sensitivity

The sensitivity and specificity of the techniques were compared using different groups of samples. The positive samples used in this experiment were divided into three types: one plasmid, four isolates (field samples that have been grown in cells), and sixteen clinical samples from FMD outbreaks. The plasmid used contained an insert with target sequences for all PCR in pTwist Amp High Copy (Molecular Brazil, Brazil). Isolate samples were obtained from FMD outbreaks in Latin America and Asia (Table 2) and multiplied in BHK21 cells. Cell suspensions were frozen, and after thawing, centrifugation was performed at 3,000 g for ten minutes. The supernatant was collected and subjected to RNA extraction.

Samples from outbreaks (collected tissues/samples with diagnoses confirmed by viral isolation) were provided by Lanagro/PA. They were all collected during the 2005 outbreak in Mato Grosso do Sul. Epithelium with characteristic lesions on the tongue and paws was

collected and subjected to viral isolation. Tissues with positive isolation for FMDV were frozen at -70°C until being used for RNA extraction in this work and were subsequently submitted to RNA extraction.

2.4 Specificity

The negative samples used in the specificity test were divided into two groups. The first group consisted of twenty-four samples with suspected vesicular disease that were sent to the laboratory and tested serologically, through viral isolation, and by RT-qPCR as is routine for the official laboratory of the Brazilian Ministry of Agriculture and Livestock. These samples tested positive in differential diagnostic tests for vesicular stomatitis, bovine papular stomatitis, pseudocowpox, and vaccinia. The second group of negative samples comprised 30 fragments of bovine epithelium without characteristic lesions of vesicular disease.

2.5 Repeatability

Repeatability tests were performed to compare FMDV.3D.107 with the other RT-qPCR techniques described in this work. The objective was to verify the response of the techniques against seven samples (tissue positive in virus isolation and RT-PCR). RNA from these samples was extracted as described above. Each of the seven samples was submitted to RT-qPCR in triplicate during three different rounds, that is, at three different times with the preparation of new reagents. The seven samples were subjected to reverse transcription and amplification, always in triplicate.

Ct values were recorded for statistical comparison. The repeatability and measurement uncertainty of each individual test were noted and then compared to FMDV.3D.107. The threshold cycle values (Ct) obtained in triplicate from each sample were evaluated considering the variations between the rounds in an analysis of variance (ANOVA) to determine the repeatability variations. Another test was carried out to verify the repeatability and reproducibility of the methods, which was an interlaboratory test. An external laboratory prepared eighteen FMDV-contaminated samples of different serotypes at various concentrations and sent them for analysis. RNA was extracted as previously described and submitted to all the techniques.

3. Results

3.1 RT-qPCR

The RT-qPCRs were standardized and compared with FMDV.3D.107, which was already in use in the laboratory. Table 2 lists the efficiency values and detection limits obtained using ten-fold dilutions of plasmid and FMDV isolates, including A24 Cruzeiro ($10^{5.5}$ TCID50/50µL), C3 Indaial ($10^{5.5}$ TCID50/50µL), O1 Campos ($10^{5.2}$ TCID50/50µL), and SAT-1 ($10^{3.9}$ TCID50/50µL). FMDV.IRES.145 exhibited the lowest efficiency, below 90%, in the performed test. Modifying the oligo concentrations did not improve the detection limit; therefore, it was excluded from subsequent tests.

Table 2 RT-qPCR efficiency and analytic sensitivity

		Plasmid		FMDV A		FMDV C		FMDV O		FMDV SAT	
RTqPCR	Efficiency	Last Positive Dilution*	Median Ct**	Last Positive Dilution	Median Ct	Last Positive Dilution	Median Ct	Last Positive Dilution	Median Ct	Last Positive Dilution	Median Ct
FMDV.3D.107	96.2%	-5 x3	39.47 (0.54)	-7 x2	40.56 (0.3)	-8 x2	40.59 (0.83)	-5 x2	39.67	-6 x2	41.06 (0.21)
FMDV.3D.88	98.7%	-5 x3	39.2 (0.34)	-7 x2	40.1 (0.8)	-7 x2	43.48 (2.46)	-6 x3	x35.99 (0.30)	-6 x3	39.75 (0.42)
FMDV.3D.99	99%	-5 x2	37.91 (1,2)	-7 x2	39.41 (0.96)	-8 x3	38.51 (2.23)	-5 x3	37.86	-7 x1	37,89
FMDV.3D.130	93%	-5 x3	37.94 (0.83)	-7 x3	37.88 (0.794)	-8 x3	39,18 (0.34)	-5 x3	39.34	-6 x1	38.81
FMDV.3D.183	91.2%	-7 x3	39.78 (0.71)	-7 x3	36.31 (1.03)	-7 x3	36.92 (0+95)	-5 x1	37.64	-6 x1	36.57

**Last positive dilution and number of positive replicates.*
*** Median Ct and standard deviation*

3.2 Specificity

FMDV.3D.88 and FMDV.3D.130 did not show any nonspecific amplification. FMDV.3D.107 amplified an epithelial sample from a bovine tongue with a Ct of 43.5. FMDV.3D.99 showed amplification with a Ct of 43.26. FMDV.3D.183 amplified five samples with Ct values ranging from 41 to 44. The observed curves did not exhibit the typical profile of qPCR curves, indicating nonspecific amplification.

3.3 Sensitivity

All samples from Foot-and-Mouth Disease oubreaks were amplified in the tested techniques, except for FMDV.3D.130. Two samples did not amplify and exhibited Ct values above 38 in the other RT-qPCR tests. FMDV.3D.88 failed to amplify one of these samples. FMDV.3D.99 consistently showed lower Ct results for all samples. The results are described in Table 3.

Table 3 Ct results for RNA extracted from ephetlium from FMDV outbreak

Sample*	FMDV.3D.107	FMDV.3D.88	FMDV.3D.99	FMDV.3D.130	FMDV.3D.183
1	26.18	26.70	25.23	26.71	25.79
2	32.03	33.04	29.72	31.04	31.06
3	35.03	35.60	32.72	34.14	34.07
4	32.79	33.05	30.92	32.12	32.11
5	27.27	27.88	25.67	26.24	26.37
6	28.30	29.10	27.02	27.96	27.76
7	26.78	26.78	25.10	26.34	25.73
8	22.72	23.31	21.13	23.25	22.13
9	23.35	23.64	21.62	24.54	22.66
10	24.38	24.91	22.89	24.51	24.79
11	22.12	23.72	20.89	23.37	22.20

12	19.92	20.33	19.23	21.79	18.31
13	24.34	24.54	23.64	26.33	24.81
14	38.50	-	38.14	-	37.38
15	38.97	39.20	38.03	-	37.38
16	40.19	40.64	39.07	39.30	39.64

** Samples from oubreaks (collected tissues/samples with diagnose confirmed by viral isolation) were provided by Lanagro/PA*

3.4 Repeatability

Repeatability tests demonstrated that all methods exhibited similar repeatability values, measurement errors, and measurement uncertainties (Table 4). Statistical tests comparing the Ct values confirmed the reproducibility of the methods among themselves. Only FMDV.3D.130 showed the highest measurement uncertainty values. FMDV.3D.107 is the RT-qPCR recommended by OIE, so the repeatability of all other methods were compared to it (Table 4). All methods had similar repeatability when samples with Ct below 33 were used in the tests. The techniques also exhibited similar efficiency in identifying negative and positive samples in interlaboratory assays. Once again, FMDV.3D.130 failed to amplify samples with higher Ct values. FMDV.3D.99 consistently amplified all positive samples with lower Ct values compared to the other techniques (Table 5).

Table 4 Repeatability tests for the five RT-qPCR and comparison of the repeatability versus FMDV.3D.107

RT-qPCR	Repeatability	Error of Measure	Uncertainty of Measure	Repeatability vs FMDV.3D.107
FMDV.3D.107	0.72	1.34	0.33	-
FMDV.3D.88	0.85	1.40	0.44	0.69
FMDV.3D.99	0.43	1.37	0.28	0.57
FMDV.3D.130	1.06	2.01	0.49	0.93
FMDV.3D.183	0.61	1.42	0.38	0.66

Table 5 Interlaboratory assay for evalutation of the RT-qPCRs tested in this study

Sample	FMDV.3D.107	FMDV.3D.88	FMDV.3D.99	FMDV.3D.130	FMDV.3D.183
1	18.79	18.20	16.45	20.21	17.71
2	19.28	20.67	16.67	20.09	18.87
3	20.76	21.20	19.27	22.96	20.25
4	26.56	26.90	24.19	28.32	26.88
5	36.98	37.50	34.02	39.39	36.53
6	36.41	36.88	34.92	36.56	36.06
7	39.47	-	38.87	-	45.08
8	-	-	38.94	-	40.50
9	-	-	37.43	-	-
10	-	-	-	-	-
11	40.52	-	38.90	-	-
12	37.76	38.53	36.13	37.86	38.74

13	-	-	-	-	-
14	-	42.00	-	-	42.00
15	-	-	-	-	-
16	27.29	27.94	26.01	26.69	26.01
17	27.06	27.55	25.77	27.27	26.16
18	-	-	-	-	-

4. Discussion

The one-step RT-qPCR is a method recommended by the OIE for detecting FMDV, offering advantages such as sensitivity and a reduced risk of contamination due to its avoidance of electrophoresis. Key attributes of RT-qPCR include speed, automation capability, sensitivity, portability, and biosafety, as it eliminates the need for viral amplification, which could otherwise increase the risk of laboratory escape. Moreover, it is a rapid method that can be partially or fully automated, thereby enhancing analysis speed and the processing capacity for numerous samples. Molecular techniques can be up to a thousand times more sensitive than viral isolation[15].

Implementing RT-qPCR in a laboratory should be approached cautiously, considering that variations in results may stem from reagent manufacturers or equipment used [15]. Consequently, performance verification and proficiency tests are imperative to ensure the correct replication of the method. Previous comparisons between PCRs have demonstrated that repeatability can be low between methods or laboratories, posing a constant risk that the routine PCR implementation may not be efficient in diagnosis or adequately standardized as per publication guidelines [16].

All the methodologies examined in this study focus on the 3D region of the FMDV, which is a highly conserved segment of the genome. Despite its conservation, this genomic area can exhibit variations that could be pertinent in the diagnostic process [17]. Verification of this fact can be accomplished by aligning the oligonucleotides mentioned in publications with sequences accessible in GenBank. Following the alignment, FMDV.3D.99 underwent modifications by incorporating primers accurately matching polymorphic regions identified in the in silico search. FMDV.IRES.145, targeting the IRES region, was also tested; however, due to low efficiency, it was excluded from further tests. One factor potentially impacting the performance of this latter method may be the generation of secondary structures in the target region, hindering the effective access of primers during reverse transcription or amplification [18].

Specificity tests revealed the occurrence of non-specific reactions in the samples used. These amplifications occurred at Ct values above 40 in epithelial samples from bovine tongues. The animals showed negative serology and viral isolation. The samples with non-specific amplifications differed among the tested techniques. It is important to note that any method can produce non-specific results. One plausible explanation for the non-specific amplifications observed in the 3D-targeted oligonucleotide tests is the potential pairing with similar regions in the Bovine Rhinitis B Virus (BRBV) genome. *In silico* specificity testing

conducted using Blast software with the entire GenBank database as the alignment target demonstrated that the primers and probes of FMDV.3D.107 exhibit 94% to 100% identity with certain BRBV sequences. BRBV can be found in nasal or tracheal swab samples and has been detected in up to 6.4% of samples tested in the United States [19]. As FMDV samples are frequently obtained from these regions, either through the epithelium or in the collection of esophageal pharyngeal fluid, the possibility of false positives increases, making it essential that these diagnoses are analyzed more carefully.

Sensitivity, interlaboratory assays, and repeatability tests were crucial in demonstrating that the methods possess similar efficiency in detecting FMDV. Nevertheless, there are slight differences, particularly the consistent failure of FMDV.3D.130 to amplify samples with lower viral load. This failure requires attention since very early or late stages of infection are associated with low viral shedding and a minimal amount of detectable viral RNA in the samples [20]. The integration of field data (clinical and epidemiological) should be combined with result interpretation.

The tests carried out in this work highlight the importance of using more than one diagnostic method in case of doubts or results with a very high Ct, which may indicate non-specific reactions. Continuous assessment is necessary, ensuring that the method effectively amplifies the samples circulating in the country, and subsequently employing the more sensitive RT-qPCR for screening purposes. Specific variants of a region may be important to the point of requiring specific oligonucleotides for circulating viruses [21].

However, it can be argued that high sensitivity may not be crucial for detecting an FMDV outbreak. According to previous publications [22], when a veterinarian attends to a suspected vesicular disease case, the clinical signs are already advanced. FMDV has the characteristic of rapidly spreading within a non-immune herd, resulting in many animals being at different stages of infection. From this perspective, a more specific method would hold greater importance.

5. Conclusion

This study underscores the importance of assessing diagnostic methods before their implementation in a laboratory setting. A comprehensive evaluation of the method facilitates a better understanding of its limitations and advantages, ultimately contributing to accurate interpretation. The potential risks associated with non-specific results highlight the need for meticulous evaluation. FMDV.3D.107 and FMDV.3D.99 emerge as the most recommended options for FMDV diagnosis based on the study's findings.

Conflict of Interest

There are no conflicts of interest to declare.

Authors' Contributions

PCR Standardization, Repeatability, Sensitivity, Specificity: Antonio Augusto Fonseca Junior

Viral Isolation:René Ribeiro da Silva

Nucleic Acid Extraction: Mateus Laguardia-Nascimento, Amanda Petrina Scotá Ferreira

Manuscript Preparation: Marcelo Fernandes Camargos, Anselmo Vasconcelos Rivetti Júnior, Antonio Augusto Fonseca Junior

References

1. Alexandersen S, Zhang Z, Donaldson AI, Garland AJ. The pathogenesis and diagnosis of foot-and-mouth disease. J Comp Pathol. 2003 Jul;129(1):1-36. https://doi.org/10.1016/s0021-9975(03)00041-0

2. Grubman MJ, Baxt B. Foot-and-mouth disease. Clin Microbiol Rev. 2004 Apr;17(2):465-93. https://doi.org/10.1128/CMR.17.2.465-493.2004

3. Amaral TB, Gond V, Tran A (2016) Mapping the likelihood of foot-and-mouth disease introduction along the border between Brazil and Paraguay. Pesquisa Agropecuária Brasileira. 2016; 51(05):661-670. https://doi.org/10.1590/S0100-204X2016000500029

4. Davies G. The foot and mouth disease (FMD) epidemic in the United Kingdom 2001. Comp Immunol Microbiol Infect Dis. 2002 Oct;25(5-6):331-43. https://doi.org/10.1016/s0147-9571(02)00030-9

5. Corbellini LG, Fernández F, Vitale E, Moreira Olmos C, Charbonnier P, Iriarte Barbosa MV, Riet-Correa F. Shifting to foot-and-mouth disease-free status without vaccination: Application of the PROMETHEE method to assist in the development of a foot-and-mouth national program in Uruguay. Prev Vet Med. 2020 Aug;181:105082. https://doi.org/10.1016/j.prevetmed.2020.105082

6. OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris. 2018.

7. Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc. 2002 Jun 1;220(11):1636-42. https://doi.org/10.2460/javma.2002.220.1636

8. Reid SM, Ferris NP, Hutchings GH, Zhang Z, Belsham GJ, Alexandersen S. Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. J Virol Methods. 2002 Aug;105(1):67-80. https://doi.org/10.1016/s0166-0934(02)00081-2 . PMID: 12176143

9. Oem JK, Kye SJ, Lee KN, Kim YJ, Park JY, Park JH, Joo YS, Song HJ. Development of a Lightcycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. J Vet Sci. 2005 Sep;6(3):207-12.

10. Dill V, Beer M, Hoffmann B. Simple, quick and cost-efficient: A universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses. J Virol Methods. 2017 Aug;246:58-64. https://doi.org/10.1016/j.jviromet.2017.04.007

11. Goris N, Vandenbussche F, Herr C, Villers J, Van der Stede Y, De Clercq K. Validation of two real-time RT-PCR methods for foot-and-mouth disease diagnosis: RNA-extraction, matrix effect, uncertainty of measurement and precision. J Virol Methods. 2009 Sep;160(1-2):157-62. https://doi.org/10.1016/j.jviromet.2009.05.005

12. El Bagoury GF, Elhabashy R, Mahmoud AH, Hagag NM, El Zowalaty ME. Development and evaluation of one-step real-time RT-PCR assay for improved detection of foot-and-mouth disease virus serotypes circulating in Egypt. J Virol Methods. 2022 Aug;306:114525. https://doi.org/10.1016/j.jviromet.2022.114525

13. Foglia EA, Lembo T, Kazwala R, Ekwem D, Shirima G, Grazioli S, Brocchi E, Pezzoni G. Combining Multiple Assays Improves Detection and Serotyping of Foot-and-Mouth Disease Virus. A Practical Example with Field Samples from East Africa. Viruses. 2021 Aug 10;13(8):1583. https://doi.org/10.3390/v13081583. PMID: 34452448; PMCID: PMC8412026.

14. Shi X, Liu X, Wang Q, Das A, Ma G, Xu L, Sun Q, Peddireddi L, Jia W, Liu Y, Anderson G, Bai J, Shi J. A multiplex real-time PCR panel assay for simultaneous detection and differentiation of 12 common swine viruses. J Virol Methods. 2016 Oct;236:258-265. https://doi.org/10.1016/j.jviromet.2016.08.005

15. Moonen P, Boonstra J, van der Honing RH, Leendertse CB, Jacobs L, Dekker A. Validation of a LightCycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. J Virol Methods. 2003 Oct;113(1):35-41. https://doi.org/10.1016/s0166-0934(03)00220-9

16. Nérette P, Dohoo I, Hammell L, Gagné N, Barbash P, Maclean S, Yason C. Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus. J Fish Dis. 2005 Feb;28(2):101-10. https://doi.org/10.1111/j.1365-2761.2005.00613.x. PMID: 15705155.

17. Moniwa M, Clavijo A, Li M, Collignon B, Kitching PR. Performance of a foot-and-mouth disease virus reverse transcription-polymerase chain reaction with amplification controls between three real-time instruments. J Vet Diagn Invest. 2007 Jan;19(1):9-20. https://doi.org/10.1177/104063870701900103

18. Nérette P, Dohoo I, Hammell L, Gagné N, Barbash P, Maclean S, Yason C. Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus. J Fish Dis. 2005 Feb;28(2):101-10. https://doi.org/10.1111/j.1365-2761.2005.00613.x. PMID: 15705155.

19. Hause BM, Collin EA, Anderson J, Hesse RA, Anderson G. Bovine rhinitis viruses are common in U.S. cattle with bovine respiratory disease. PLoS One. 2015 Mar 19;10(3):e0121998. https://doi.org/10.1371/journal.pone.0121998. PMID: 25789939; PMCID: PMC4366061.

20. El Bagoury GF, Elhabashy R, Mahmoud AH, Hagag NM, El Zowalaty ME. Development and evaluation of one-step real-time RT-PCR assay for improved detection of foot-and-mouth disease virus serotypes circulating in Egypt. J Virol Methods. 2022 Aug;306:114525. https://doi.org/10.1016/j.jviromet.2022.114525. Epub 2022 Mar 23. PMID: 35337855.

21. Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL. Comparative genomics of foot-and-mouth disease virus. J Virol. 2005 May;79(10):6487-504. https://doi.org/10.1128/JVI.79.10.6487-6504.2005

22. Stenfeldt C, Eschbaumer M, Rekant SI, Pacheco JM, Smoliga GR, Hartwig EJ, Rodriguez LL, Arzt J. The Foot-and-Mouth Disease Carrier State Divergence in Cattle. J Virol. 2016 Jun 24;90(14):6344-64. https://doi.org/10.1128/JVI.00388-16

23. Keck H, Hoffmann B, Eschbaumer M. Proof of Proficiency of Decentralized Foot-and-Mouth Disease Virus Diagnostics in Germany. Viruses. 2022 May 20;14(5):1098. https://doi.org/10.3390/v14051098

24. Shi X, Liu X, Wang Q, Das A, Ma G, Xu L, Sun Q, Peddireddi L, Jia W, Liu Y, Anderson G, Bai J, Shi J. A multiplex real-time PCR panel assay for simultaneous detection and differentiation of 12 common swine viruses. J Virol Methods. 2016 Oct;236:258-265. https://doi.org/10.1016/j.jviromet.2016.08.005

25. Xu X, Yang F, Zhang Q, Xu Y, Huang J, Fu M, Zhang W. Development of a multiplex TaqMan qPCR assay for simultaneous detection and differentiation of four DNA and RNA viruses from clinical samples of sheep and goats. J Virol Methods. 2019 Apr;266:58-64. https://doi.org/10.1016/j.jviromet.2019.01.015

26. Rasmussen TB, Uttenthal A, de Stricker K, Belák S, Storgaard T. Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of foot-and-mouth disease virus. Arch Virol. 2003 Oct;148(10):2005-21. https://doi.org/10.1007/s00705-003-0145-2

27. Wernike K, Hoffmann B, Beer M. Single-tube multiplexed molecular detection of endemic porcine viruses in combination with background screening for transboundary diseases. J Clin Microbiol. 2013 Mar;51(3):938-44. https://doi.org/10.1128/JCM.02947-12