From Virus to Disease: Exploring the Role of the Causative Agent in Foot Mouth Disease

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Abstract

Foot-and-mouth disease virus (FMDV) is the causative agent of an acute infection that affects animals with cloven hooves, such as sheep, goats, cattle, and pigs. The infection poses a serious risks to both animal husbandry and international commerce of animals' products. FMDV is a member of the Aphthovirus genus within Picornaviridae family. It has a non-enveloped icosahedral capsid with a diameter of 25–30 nm, enclosing a genome made of single stranded RNA that is a positive sense orientation and an approximate size of 8.4 kb. The virus are divided into seven serotype that are distinct from each other; A, O, C, Asia-1, SAT1, 2, 3. However, within each serotype, many subtypes have emerged over time. This Review explores the genetic, structural, antigenic, and epidemiological features of FMDV. This will help to investigate how FMDV interacts with its host and how to devise effective strategies for its prevention and control.

Keywords: FMDV, structure, serotypes, epidemiology

I. INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and acute viral infection that affects practically all cloven footed domesticated mammals including cattle, buffalos, sheep, goats and pigs and many wild herbivores. Infected animals develop fever, blisters and ulcers on their mouth, feet, and mammary glands. The infection can spread rapidly through various routes, such as direct contact, aerosols and fomites. FMD has caused several outbreaks worldwide, resulting in enormous governmental and financial victims for humans. The first record of cattle with FMD dates back to 1514 by an Italian monk, H. Fracastorius. In 1897, the causative agent of FMD was identified as a virus (FMDV) by Loeffler and Frosch. (1). FMD disease is caused by a virus that is part of the Picornaviridae family and the Aphthovirus genus. This Review explores the genetic, structural, antigenic and epidemiological features of the virus.

FMDV GENETIC FEATURES

The FMDV genome spans about 8.5 kilobases and comprises a single-stranded RNA with a positive polarity (2). Divided into three segments, it features a 5′ non-coding region on the left with a small viral protein VPg (or 3B) that is covalently bonded. It is an open reading frame (ORF) in the center and 3′ non-coding region on the right. The 5′ end of the mRNA has a region that does not codes for proteins specifically includes a small viral protein VPg (or 3B) linked through a covalent bond. The 5′ untranslated region (UTR) of FMDV contains several structures, Examples of these elements encompass the S-fragment (a concise portion of the genome), the poly© tract, the pseudoknot, the cre element, and the internal ribosome entry site (IRES) (3).

The S-fragment has the capacity to create an extended stem-loop configuration, distinctly set apart from the remainder of the genome by a series of cytosine nucleotides known as poly©. Variations can be observed among the S-fragments across various serotypes of FMDV (4). Carrillo et al. found that the S-fragments had 80% sequence similarity, showing that they were very conserved (5). The newly synthesized RNA is protected by the S-fragment from being degraded by nucleases and ensure the replication process of the viral RNA. The S-fragment also theatres a part now modulating the distinctive immune system. Kloc et al. showed that deleting additional than 163 nt from the stem-loop of the S-fragment made the viral RNA unstable (6). Moreover, a single mutation of G320T in the S-fragment prevented the recovery of viable virus (7).

Following the poly© tract are three or four reiterated pseudoknots (PKs) (9). In a recent studies involved scientist creating PK recombinant FMDV strains to measure the virulence and pathogenicity of various FMDV strain in pigs and cattle. Their findings revealed that the absence of varied sizes of PKs influenced the host's reaction to the virus, underscoring the significance of the pseudoknot region in determining FMDV's viral tropism and virulence (10).

A type of structure named (cre) cis-acting replication element which affect replication, is present in the coding region of some picornaviruses. The cre is conserved AAACA sequence and form a loop that is connected by double stranded stem. The addition of U residues to the protein primer 3B is beneficial (11). Mason et al. discovered the indispensability of the cre in genome replication, noting its ability to operate effectively irrespective of its placement at the 5′ end of the genome (12).

Eukaryotic cells usually start translation by recognizing a cap structure at the 5′ end of mRNA. Yet, picornavirus RNAs possess an alternative translation initiation method, utilizing an operational component known as the internal ribosomal entry site (IRES) located at the 5′ end of the mRNA (13). The FMDV IRes, consisting of four-hundred sixty two nucleotide and divided into 5 domains (14), initially demonstrated the crucial interaction between the IRES and the translation initiation factor eIF4G. This interaction, acting as a bridge during translation initiation, was found to be vital for in vivo translation (15). Subsequent studies unveiled additional interactions of the FMDV IRES along three other translation initiation factor eIF3, eIF4B, eIF4GII (16). Moreover, IRES trans-acting factors (ITAFs) play a pivotal role in promoting IRES-mediated translation across all cell types. Notably, the host range of FMDV does not affect the IRES- mediated translation activity and stays independent of it, with observed effects limited to polypyrimidine in a cell that susceptible to FMDV tract binding protein (PTB) and a protein that binds to eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) interact with each other (17). Additionally, domain binding protein 1 (G3BP1) Ras GTPase SH3 directly binds to the FMDV IRES, exerting a negative regulatory impact on translation (18).

The FMDV genome has a single long ORF that codes for four proteins that form the virus structure (VP1, VP2, VP3, and VP4) and 10 protein that are not part of the virus structure (L, 2A, 2B, 2C, 3A, 3B1, 3B2, 3B3, 3C, and 3D). These diverse proteins play distinct roles in the virus's life cycle, each of which will be detailed in subsequent sections (19, 20).

At the FMDV genome 3′ end, there exist two components: a 90-nucleotide non-coding region (3′-NCR) and a poly(A) tail. These elements collaborate to bolster the translation of the viral RNA facilitated by the IRES. (21). The IRES, positioned at the 5′ end of the viral RNA, establishes an interaction between the 3′ and 5′ prime ends of the virus. Serrano et al. demonstrated the involvement of certain elements within the 3′ UTR in mediating connection or interaction between the IRES and the S region. This observation suggests direct contact between the RNA's 5′ and 3′ ends, playing a role in RNA replication (22). The absence of SL1 and SL2, two specific stem-loop structures within the 3′-NCR, significantly impacts virus infectivity. The ΔSL1 mutation renders the virus non-pathogenic to pigs yet elicits an immune response crucial for developing FMDV vaccines (23). Additionally, SL2 emerges as a pivotal component essential for virus replication (23, 24).

II. STRUCTURAL PROTEINS

Four proteins make up the shell of FMDV, an enveloped virus with a spherical shape: VP1, VP2, VP3, and VP4. The outer layer of the shell consists of VP1, VP2, and VP3, while VP4 is inside the shell, touching the RNA strands (25). Malik et al. used a technique called cryo-EM to get a high-resolution picture of the FMDV shell, but they did not see VP4 in their picture (26). At low pH and elevated temperatures, FMDV shells exhibit sensitivity and fragment into pentagonal pieces, subsequently discharging RNA (27).

Barteling and colleagues posited in 1982 that the FMDV protein VP1 could play a role in the initial phase of cell- virus interaction (28). The next year, Dawe and king found that the early and late version of the virus had different abilities to infect cell and mice, and they found that the changes in VP1 were the reason for this (29). As the majority of VP1 is prominently displayed on the virus surface, it governs the recognition of the viral particle by the immune system. VP1 can enable the body to produce specific antibodies that can block the virus and protect against infection (30–34). VP1 has a loop that contains a sequence of three amino acids: RGD. The virus uses this sequence to stick to the cell (35, 36). Based on this feature of VP1, many efforts have been made to create an FMDV vaccine (33, 37–42). Also, studies show that the stability of the FMDV shell in acidic environments is related to the N-terminal part of VP1 (43). New research revealed that VP1 can block beta interferon signaling pathways by preventing the activation of IRF3, a key protein for the immune system. However, this effect can be reversed by another protein, DNAJA3 (44).

The persistence of FMDV is associated with the protein VP2, according to researcher (45). Alteration in the amino acids in the B-C loop of the VP2 protein cause the antigens of different FMDV types to differ, implying that VP2 plays a part in the antigenic diversity of FMDV (46). Furthermore, VP2 affects the virus's ability to reproduce and its severity (46).

As Vazquez-Calvo et al. reported (47), a tyrosine substitution for a histidine in VP2 enhanced the FMDV shell's resilience to low pH. Subsequent studies showed that VP2 was involved in triggering the EIF2S1-ATF4 pathway in cells and stimulating autophagy via the HSPB1 protein [48]. Furthermore, VP2 was found to have potential uses in both vaccine creation (49, 50) and viral typing (51–53).

VP3 is another shell protein of FMDV. A mutation that removed an amino acid at the 59th position of VP3 protein was observed in FMDV from India, and this change enhanced the disease transmission (54–56). Moreover, substituting a histidine with an aspartic acid in VP3 made type A FMDV more resilient to low pH (57). FMDV VP3 inhibited both the IFN-beta and IFN-gamma signaling pathways, as reported by studies (58, 59). Qi et al. discovered that a host microRNA, named miR-1307, assists in degrading the viral protein VP3 through the proteasome mechanism. This finding suggests a potential application for the therapy of foot-and-mouth disease. (60).

T cell epitopes are recognized by the immune systems T cells via MHC molecules, which are essential for immunity. FMDV VP4's segment from 20 to 35 could play a role in this process. This characteristic could be useful for developing peptide vaccines (61,62).

III.THE FMDV'S NONSTRUCTURAL PROTEINS

FMDV has an ORF in its genome, which codes for two forms of a protein called L-pro, which is the first protein that the virus makes. L-pro can be made from two different start codes: AUG. The first AUG makes Lab, and the second AUG makes Lb (63). The virus could still produce new viruses within cells without the 1st AUG, but not without the 2nd AUG, as scientists found out (64). FMDV stops the production of proteins in host cells after infection, a process that may be related to the cutting of a protein called eIF4GII by L-pro (65). L-pro can increase translation through the IRES showed by Moral-Lopez et al (66). Also, researchers compared L-pro sequences from different FMDV types in India and saw the same amino acids at the site where L-pro cuts. (67).
The P2 region of FMDV is processed to produce three different proteins: 2A, 2B, and 2C [68]. The 2A protein of FMDV has the ability to cleave at the junction between 2A and 2B, a characteristic that researchers exploit to produce multiple proteins within cells for biotechnological applications (69–76). This cleavage occurs due to a preserved amino acid sequence that all FMDV share: [D(V/I)E(S/T)NPGP], where the final P initiates 2B and is crucial for both protein processing and virus replication (77, 78). Trials producing the FMDV P1-2A3C protein in plants demonstrated its potential to induce antibody formation in guinea pigs (79). Moreover, targeting FMDV's 2A protein with a vaccine is a hopeful strategy to manage foot-and-mouth disease (80, 81). Zhu et al. revealed 2B's function in reducing the expression of the RIG-I protein by binding with amino acids 105–114 at the terminus of 2B (82). Subsequent studies indicated 2B's interaction with MDA5, inhibiting IFN-beta production (83). Additionally, research showed 2B's activation of the NLRP3 inflammasome protein complex (84). Further studies detected interactions between FMDV's 2B protein and eEF1G [85] and CypA (86), emphasizing its significance in virus infection and replication. 2C is a tool to distinguish between infected and vaccinated animals (87–90). Researchers identified proteins that interact with 2C, such as Beclin1 (91) and Nmi (92, 93), using techniques like yeast two-hybrid assays and immunoprecipitation to elucidate FMDV mechanisms.

3A was also used to tell apart infected and vaccinated animals (94), being more specific and sensitive than 3B and 3AB (95). Gladue et al. saw the effect of the interaction between 3A, and a host protein called dctn3 on the virus virulence (96). A 2013 study said that removing a part of 3A made the virus weaker in cattle (97), but later research after five years said that this removal didn't stop infection without symptoms (98). The FMDV genome has 3 copies of the 3B protein (or VPg), and 3A was shown to inhibit interferon-Beta signaling to avoid the host immune system (99). Studies showed a link between the number of 3B copies and viral virulence, with a virus having only one 3B being less virulent and causing less severe disease
(100). The 3C protease cuts the FMDV shell precursor P2-2A into VPO, VP3, VP1, and 2A, one by one. (101). 3C protease is a protein that cuts other proteins. Birtley et al. got a very clear picture of 3C protease using crystals, and they saw that it had a shape like chymotrypsin and had three amino acids that were important for cutting: cys-his-asp (102). Researchers showed that 3C also cuts the host cell skeleton when FMDV infects the host (103).

The 3C protease can stop autophagy by breaking down the ATG5-ATG12 protein complex (104), as more studies have shown. The last non-structural protein, 3D polymerase, makes more RNA from the viral RNA. Scientists have shown that making microRNAs that target 3D polymerase can effectively block FMDV replication in cells (105, 106). So, 3D polymerase is a good target for making antiviral drugs against FMDV. For example, 5d9, a 3d polymerase

inhibitor, can stop FMDV replication process in host cells well (107). However, many questions remain, and more research is needed to understand the roles and mechanisms of FMDV non-structural proteins.

IV. DIFFERENT VARIANTS OF FOOT-AND-MOUTH DISEASE VIRUS

Foot-and-mouth disease virus has seven main types: A, O, C, Asia-1, SAT 1, SAT 2, and SAT 3, each with several subtypes. The disease has impacted many regions of the world, and type O is the most prevalent. Outbreaks in Africa have been caused by six types (A, O, C, SAT1, SAT2, and SAT3), in Asia by four types (O, A, C, Asia1), and in South America by three types (O, A, C) (108). Viruses of SAT 1 and SAT 2 types from Africa have also reached the Middle East (108). The type C virus caused the last outbreak of foot-and-mouth disease in 2004, and it is assumed to have vanished since then. (109).

EVIDENCE OF FMDV EPIDEMIOLOGY

FMDV is a highly contagious and rapidly spreading virus that infects animals with split hooves, such as cattle, swine, sheep, goats, camels, and wildlife. The virus can be transmitted by animals that have symptoms of the disease or are in the incubation period, which ranges from 1 to 7 days, typically 2 to 4 days. The virus can be found in various secretions and excretions of infected animals, such as blisters, milk, urine, saliva, tears, and feces. Animals that lack immunity to the virus can get infected by direct contact. (for example, touching noses) or indirect contact (for example, breathing polluted air, eating contaminated food, using infected animal products, etc.). The disease is more common in spring and fall, and it usually causes blisters in the mouth, feet, and udder. Foot-and-mouth disease has caused many outbreaks around the world, resulting in serious political and economic consequences and losses.

V. CONCLUSION

Foot-and-mouth disease is a serious threat to animal farming, as it lowers the milk production of infected animals, causes death in severe cases, and leads to huge economic losses. That is why many countries regard foot-and-mouth disease as a major animal quarantine issue. However, the disease is still widespread in some developing countries, making it hard to prevent and control, mainly because of the existence of seven different serotypes of the FMD virus, each with specific antigens that do not offer cross-protection. Vaccination is a trustworthy and effective way to protect specific populations from FMD, requiring the creation of a safe and potent vaccine that is crucial for stopping, controlling, and ultimately eliminating the disease. Therefore, comprehensive research on the virus's mechanisms and the creation of more effective prevention and control methods are essential to ensure the healthy growth of animal farming.

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