Korean Journal
of Veterinary Service

Korean J Vet Serv (2004) 27(4) : 379~391

Foot and mouth disease : literature review

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(Received 30 March 2004, accepted in revised from 12 July 2004)

The virus

The first written description of foot and mouth disease (FMD) probably occurred in 1514, when Fracastorius described a vesicular disease of cattle in Italy. Almost 400 years later, in 1897, Loeffler and Frosch demonstrated that a filterable agent caused FMD. This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology[1]. Subseq uently it was shown that the agent, FMD virus (FMDV) consist of a single-stranded, plus-sense RNA genome of approximately 8,500 bases surrounded by four structuralproteins to form an icosahedral capsid[2]. FMDV is a virus of the Aphthovirus genus, family Picorna rviridae. Seven serotypes, namely A, O, C, Asia 1, SAT 1, SAT 2 and SAT 3 have been identified serologically, and multiple subtypes occur within each serotype[3].

FMD is currently present in all continents except Australia and North America. Economically FMD is the single most important infectious disease of cattle, pigs, sheep, goats and wild cloven-hoofed animals, thereby the disease is classified as the list A of infectious diseases of the Office International des Epizooties (OIE) and has been recognized as the most important constraint to international trade in animals and animal products[4, 5].

FMD is one of the most highly contagious diseases of animals. FMDV rapidly replicates in the infected animal, and spreads to the susceptible animals by contact and aerosol means. Clinical signs can appear within 2 to 3 days after

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FMDV exposure, and last for 7 to 10 days. The disease is characterized by fever, inappetence, and decrease in milk production, lameness and vesicles on feet and teats and also in the mouth. Usually, mortality in the infected adult animals is negligible, but severe impairment of production, slaughter of the affected animals and interference with international trade results in severe economic losses. Nevertheless, mortality in young animals may be considerably high[6-10].

Genome organization

The genome of FMDV, which is over 8000 bases in length, is covalently bound to a 23-24 amino acid residue of genome-linked protein, 3B at its 5'-termius. In the mature virus, the genome is encapsidated in an icosahedral structure consisted of 60 copies of four structural proteins (VP1, VP2, VP3, and VP4)[11-13]. The viral RNA consists of a single open reading frame (ORF), flanked by two highly structured non-coding regions (NCRs) that contain cis-acting structural elements involved in viral replication and gene expression. A small viral protein, VPg, is covalently linked to the 5'end of viral RNA. The 5' NCR (about 12 nt) is divided by a poly (C) tract. The translation initiation of the FMDV RNA starts at two AUG codons, following ribosome recognition of the upstream internal ribosome entry sequence (IRES), which spans the 465 nt preceding the first functional AUG[14, 15]. The IRES element, present in all picornaviruses, provides a cap-independent translation function[16]. A highly structured region of about 90 nt is also predicted at the 3'NCR of the FMDV genome, preceding a genetically encoded poly (A) tract. This region, for which there is extensive evidence of its interaction with several viral and host proteins in other picornaviruses, is essential for viral replication[17].

Gene expression

Replication and translation of FMDV RNA occur in the cytoplasm of infected cells, in association with cell membranes. FMDV RNA is infectious by itself when transfected into susceptible cells. This feature has allowed the construction of infectious cDNA clones, which are being used to study on the function of different genes and RNA structural motifs[15 18].

The genome contains a single long open reading frame (ORF), that has two alternative initiation sites, and the encoded polyprotein which is cleaved by viral protease into over a dozen well-described mature polypeptides as well as a variety of partial cleavage intermediates (Fig 1). The L region contains two overlapping proteins, Lab and Lb, which result from the translation initiation from each of the two functional AUGs of the polyprotein[12, 19]. The P1-2A region encodes the structural proteins VP1, VP2, VP3 and VP4. The 16 amino acids peptide 2A catalyses, in cis, excision of the P1-2A from 2B[20]. The P2 and P3 encode six different mature non-structural proteins.

Each of them, as well as some of the processing intermediates, is involved in functions relevant to the virus cycle in the infected cells[21]. A helicase domain is present in 2C, in which of viral RNA replication, have been reported[22], supporting its implication in viral RNA synthesis. The polypeptide 2C and its precursor, 2BC are associated with cell membranes, and induce a vesicle proliferation[23]. 3A is likely to play a relevant role in the pathogenesis of FMDV in natural hosts, since changes in 3A, reported for several FMDV1 strains and serotypes, are associated with attenuation for cattle[24]. VPg participates in the initiation of picornavirus RNA synthesis. In FMDV, the only picornavirus containing three tandem, non-identical copies of 3B (VPg), the level of viral infectivity correlates with the number of copies present in the RNA[25]. 3C is a thiol-protease responsible for most of the cleavage events undergone by the viral polyprotein. 3C also induces the proteolytic processing of histone H3, which may relate to the inhibition of host transcri ption observed in infected cells[26]. The 3D protein is the viral RNA-dependent RNA polymerase[13]. The error-prone replication of FMDV RNA results mostly in point mutations, which arise at frequencies of around 10-4nucleotide substitutions per site. RNA recombination can also occur during viral replication, and FMDV was the first RNA virus in which in vitro recombination was described[27].

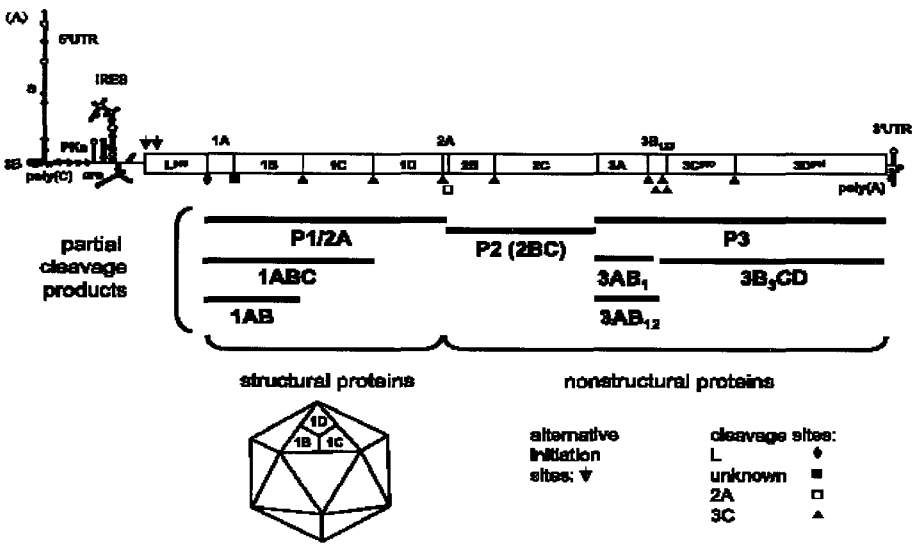

Fig 1. Schematic map of the FMDV genome.
The ORF is shown in the boxed area, with the viral proteins named according to the nomenclature of Rueckert and Wimmer[28]. Also shown are the functional elements of the genome as described in the text and the partial protein cleavage products. The sites of the primary cleavages and the proteases responsible are indicated.

Structure and properties

The structural precursor P1 is rapidly cleaved into protein VP0 (precursor of VP4 and VP2) and VP3, which assemble into asymmetric units or protomers. Five protomers associate to form a pentamer, and 12 pentamers incorporate a newly synthesized RNA molecule to conform a virus particle (Fig 1)[12]. VP2 and VP4 are produced, following RNA encapsidation, as a result of the autocatalytic cleavage of VP0. The structure of FMDV virion corresponding to serotypes O, A, and C has been elucidated by X-ray crystallography[29]. FMDV capsids show the classic structural organization of the picornavirus family. They consist of non-enveloped capsids of icosahedral symmetry, 28-30nm in diameter, composed of 60 asymmetrical protomers (Fig 1) in which VP1, VP2 and VP3 are surface-oriented, whereas VP4 is internal-oriented and has an N-terminal myristic acid[30]. VP1-3 share a similar structural pattern.

Despite showing structural similarities with other picornaviruses, FMDV exhibits distinctive features. Unlike most picornaviruses, FMDV capsid surface is relatively smooth, exhibiting a main protruding element, the G-H loop, in VP1. The G-H loop spans about 20 residues around without apparent perturbations of the rest of the capsid. This large loop contains a highly conserved Arg-Gly-Arp (RGD) triplet, a universal cell-recognition site present in various extracellular proteins, which interacts with cell surface integrin receptors.

The antigenicity of FMDV particles is associated with amino acid residues that are well exposed on the surface of the capsid[31, 32]. A major continuous antigenic site is located in the G-H loop of VP1, as deduced from the immunogenicity of peptides spanning VP1 residues around positions 140-160[33, 34]. In addition, a large proportion of monoclonal antibody (mAb) -resistant mutants obtained with mAbs raised against entire viral particles include amino acid substitutions within this site. For serotype C, the antigenic structure of the G-H loop is complex, since different overlapping epitopes, defined by their differential ability to react with individual mAbs, have been mapped within this loop. Thus, the G-H loop is involved in both the antigenic properties of FMDV and its interaction with host cells.

Two additional neutralization sites have been described by analyzing mAb-resistant mutants. Site C is located at the C-terminus of VP1 and is apparently continuous and independent from the G-H loop in serotypes A and C[35]. In type O, its vicinity to the G-H loop in the structure of the capsid, as well as competition studies with neutralizing mAb, suggests that sites A and C conform a single site composed of discontinuous epitopes. In serotype C, site D is discontinuous, comprising residues invoving the C-terminus of VP1, the VP3 B-B knob, and VP2 B-C loop[35]. These antigenic sites are located at exposed regions adjacent to each other and close to the threefold axis of symmetry in the capsid.

Epidemiology

FMDV produces an acutesystemic

vesicular disease, which requires a differential diagnosis from other vesicular diseases. It affects cloven-hoofed animals, including cattle, swine, sheep and goats, and more than 30 species of wild ruminants. In natural infections, the main route of virus entry is the respiratory tract. The initial virus multiplication usually takes place in the pharynx epithelium, producing primary vesicles, or "apthae"[36]. The clinical outcome of the disease may vary among the host species considered and the infecting virus strain. In cattle and pigs, fever and viraemia usually start within 24-48 hours after epithelium infection, leading to viral spread into different organs and tissues, and the production of secondary vesicles preferentially in the mouth and feet. The acute phase of disease lasts about 1 week and declines gradually coinciding with the emergence of a strong humoral response[37]. In sheep and goats, symptoms are frequently less severe and may make the detection of the disease difficult. Mortality can be observed among young animals, often associated with lesions in the myocardium. The vesicles produced by FMDV generally affect cells from epithelium stratum espinosum. A high viral amplification frequently takes place in infected animals, mostly in pigs, for which up to 1012 infection units per infected animal have been scored[38]. An asymptomatic, persistent infection can be established in ruminants, during which infectious virus can be isolated from the oesophagus and throat fluids of the animals from a few weeks up to several years of the initial infection[37]. There is epidemiological evidence to support the hypothesis that carrier animals may be the origin of outbreaks of acute disease when brought into contact with susceptible animals. This mode of transmission has been experimentally reproduced for serotype SAT isolates[39].

FMD is a highly transmissible disease, and a limited number of infective particles can initiate host infection[38]. Contaminated animal products, non-susceptible animals, agricultural tools, people and vehicles can contribute to the mechanical dissemination of FMDV[40]. The epidemiology of FMD is complex, and it is affected by different viral, host and environmental factors. FMDV multiplication and spread also depend on the host species, nutritional and immunological status, population density, animal movements and contacts between different domestic and wild host. The environments can provide geographical barriers to virus dissemination or, altern atively, can promote virus transmission when appropriate atmospheric conditions prevail. In this multifactorial scenario, the high potential for FMDV variation and adaptation has modeled complex evoluti onary patterns that are being revealed by molecular epidemiology analyses, mostly based on nucleotide sequencing of capsid protein genes[13, 41].

Host cell interaction and pathogenesis

A first requirement for FMDV infection is the interaction of the virus with receptors present in host cells. Structural and functional studies with FMDV over the last decade have established that the G-H loop of capsid protein VP1 and, particularly, its RGD motif are critically involved in virus interaction with integrin

αvβ3 and other related integrin in cell culture and cattle[42-44]. However, remark able changes in receptor specificity have been recently documented for FMDV as a result of virus evolution in cell culture. Viruses propagated in cell culture can exploit alternative pathways, independent of integrin-binding, to recognizeand enter cells. Alterative receptors for FMDV include heparan sulfate and other unidentified cell surface molecules[45, 46].

Little is known of changes in receptor specificity and antigenicity accompanying changes in host range in nature, but there is no reason to rule out that emerging and re-emerging FMDVs could be endowed with such unusual biological properties. A recent study analyzing the genetic changes selected during adaptation of FMDV to guinea pig documented the progressive dominance of an unusual amino acid replacement (L147P) affecting the antigenic structure of the G-H loop of capsid protein VP1 in the course of adaptation of FMDV to this new host[47]. Experiments using an FMDV infectious cDNA confirmed that this mutation was essential for virus interaction with integrin receptor molecules expressed in BHK cells and various other cell lines commonly used to propagate FMDV.

The isolation of FMDV mutants displaying altered cell tropism in association with antigenic changes from different animal species, commented on above, illustrates the important adaptive potential of FMDV and the capacity of this virus to explore new antigenic/ receptor recognition structures upon replic ation in the host.

The virulence caused by FMDV may vary depending on the species affected as well as the dose and genomic characteristics of the infecting viral isolate[11, 36, 48]. The concept of host range is mostly based on the capacity of FMDV to induce clinical symptoms, and absence of those symptoms does not exclude the potential of FMDV to replicate in a given species. Adult mice do not develop lesions; however, they develop fever, and infective viruses can be isolated from circulating blood. Little is known about virulence and hos-range determinants of FMDV in vivo. As discussed previously, interaction of RGD triplet on VP1 with cell integrin is considered a requirement for virus entry. Nevertheless, integrin are expressed in a variety of tissues and species, including those considered non-permissive for FMDV infection. Evidence has been recently obtained on the involvement of non-structural proteins on viral virulence and host tropism. Deletions in the non-structural protein 3A were associated with attenuation for cattle of FMDV serotypes O and C, upon passages in chicken embryos[49]. An overlapping 10-amino acid deletion together with different point mutations in 3A have been shown to contribute to the low virulence for cattle of a variant of FMDV serotype O, isolated in Taiwan in 1997[24]. Interestingly, this variant virus showed a highly virulent character for swine, which illustrates the species-specificity often associated with FMDV attenuation, and the complex interactions that take place between the host and the virus that determine the progress of the infection towards the emergence of lesions and clinical symptoms.

The analysis of the molecular basis of the adaptation of FMDV to the guinea pig

has also provided evidence on the role of protein 3A in the host range of this virus. Viruses that acquired the capacity to produce fever and lesions in the guinea pig exhibited single amino acid replacement in protein 2C, 3A and VP1. The use of recombinant viruses harboring these mutations revealed that the single amino acid replacement in 3A was sufficient for FMDV C-S8c1 to produce pathology in guinea pigs[49].

Current evidence on FMDV and other picornavirues indicates that the interaction of the FMDV quasispecies with different hosts results in complex dynamics driven by different selective pressures. Among them are the requirement for cell receptors to initiate infection in different target tissues, the need to replicate to a minimal extent to allow virus progression, and the evasion of the host immune response. Mutations emerging in response to these selective pressures can affect multiple sites on the viral genome, including structural and non-structural proteins.

Diagnosis of FMD

The earlier use of complement fixation test has largely been supplanted by ELISAs due to its sensitivity, specificity and ability to deal with large number of samples. Laboratory diagnosis is usually made by ELISA detection of specific FMDV antigen in epithelial tissue suspension, often accompanied by concurrent cell culture isolation and the application of ELISA to any samples showing a cytopathic effect[50-53]. These tests are used to confirm the clinical diagnosis and to identify the FMDV serotype. Recently, RT-PCR assays have been developed for the diagnosis of FMDV infection. Although various procedures for conventional RT-PCR have been published, none seems to be of sufficient sensitivity, specificity and robustness for diagnostic work, unless supported by the other techniques[54-56]. More recently, fluorogenic real-time RT-PCR methodology as an FMD diagnostic tool was developed and evaluated. This method combined the total RNA extraction and RT procedures of conventional RT-PCR with PCR amplification by means of a fluorogenic probe in real-time RCR equipment. This assay has been used on a large number of tissue samples, serum samples, swab samples and tissue culture supernatants and more recently on probang samples, all with encouraging results.

1. Test for antigen/virus/genome

The current techniques used for FMD diagnosis are highly sensitive and specific. The type of sample recommended for testing is based on the amount of virus present in various tissues, excretions and secretions. For many of the tests, especially in ELISA methods, vesicular epithelium or vesicular fluids is preferred, as these materials contain high titers of FMDV. The more sensitive methods, such as cell culture and RT-PCR, are appropriate for samples likely to contain smaller amounts of infectivity or viral RNA.

For most FMDV strains the BTY cell system is about 10 times more sensitive than other cultures[36, 57]. However, certain

pig-adapted strains (Taiwan 1997 strain) grow more easily in IBRS-2 cells[58]. Virus isolation in BTY an IBRS-2 cell culture essentially detects all positive samples with more than 1-5 infectious units per ml or per 0.1 g.

Real-time RT-PCR methods also developed and are as sensitive as the combined ELISA/virus isolation system, detecting essentially the same positive samples as those detected by virus isolation, with high sensitivity and specificity, and providing a definitive result on the day of sample receipt.

2. Antibody detection

The liquid phase blocking ELISA (LPB-ELISA) was routinely used for FMD antibody detection until recently[59, 60]. Samples giving inconclusive results are tested by a virus neutralization test (VNT)[61]. The relatively low specificity of the LPB-ELISA makes the method less than optimal for large-scale screening purposes, as numerous confirmatory VNTs are likely to be required. Recently, a solid phase competitive ELISA (SP-C ELISA) has been developed, validation tests showing high sensitivity and specificity (99.8) at the chosen "cut-off"[62]. This method detects all experimentally infected animals at 5-8 days after infection and for several months thereafter.

The VNT is used to investigate inconclusive antibody results obtained by ELISA. The specificity of the VNT at a dilution of 1 in 45 is 100%. At present the VNT is recommended by the OIE as the definitive "gold standard" for the final assessment of such results. It is possible that when sufficient validation data become available, some of the newer tests may replace the VNT.

3. Distinguishing infected from vaccinated stock

At present there is no fully validated, OIE-approved test capable of making this distinction. Although probang sampling can identify carrier animals,it cannot be used to exclude the possibility of carriers because amounts of FMDV in the samples are low and decline over time, and also the excretion of virus by carriers is intermittent[63]. Moreover, no statistical sample frame can reliably demonstrate the absence of infection. Indeed, such a programme would probably be impracticable for large-scale surveillance, due to the amount of work required. In addition, the testing of antibodies to FMDV nonstructural proteins gives no absolute guarantee of freedom, as a significant proportion of vaccinated carrier animals may fail to demonstrate an anti-NSP response[64]. However, this test could fully validate according to an established and statistically valid sampling frame.

4. Tests to detect antibodies against the conserved, non-structural protein (NSPs) of FMDV

Such tests have been developed in several laboratories[64-70]. These methods can be used to distinguish infected animals from vaccinated animals on a herd basis, but separate assays are required to test ruminants and pigs. Initial laboratory results are encouraging but,

despite the recent successful development by some manufacturers of vaccines from which NSPs have been removed, more works is required to demonstrate that they satisfy validation criteria under field circumstances. Furthermore, in relation to carriers, the problem remains that some vaccinated carrier animals fail to develop antibodies against the NSPs, even though they are carrying live virus in their pharynx [64]. Thus, at present, tests for antibodies to NSPs cannot fully guarantee that a population of vaccinated animals exposed to live virus contains no carriers. In contrast, a non-vaccinated population can be screened by tests which detect antibodies to structural antigens, and a negative result will exclude FMDV infection in a statistically robust manner.

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