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# A Review on Genetic and Antigenic Characterization of Foot and Mouth Diseases

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**Abstract:** A large variety of domestic and wild animals with cloven hooves are susceptible to the highly contagious viral disease foot-and-mouth disease. It is still prevalent throughout the world and is one of the most economically significant livestock diseases. The virus is infamous for its extreme genetic and antigenic diversity. Through virus isolation and molecular techniques like rRTPCR, BLASTn, BLASTp and MEGABLAST, as well as the use of diagnostic tools, the genetic variation among the serotypes is identified. The most popular diagnostic techniques for antigenic characterization are liquid-phase blocking ELISA and VNT. This variation happens as a result of homologous recombination between two strains of the foot-and-mouth disease which results in the creation of new virus variants. These new variations will significantly affect the choice of foot and mouth disease.

**Abbreviations:** AA amino acid; *BLAST* -basic local alignment search tool; *LPBE* = liquid phase blocking ELISA; *RT-PCR* = reverse transcriptase. Polymerase chain reaction

**Key words:** Antigenic and Genetic Characterization · FMDV · Vaccine Matching

## INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and devastating viral disease affecting a wide range of domestic and wild cloven-hoofed animals [1]. The causative agent of FMD is the foot-and-mouth disease virus, a member of the genus *Aphthovirus inn* the family *Picornaviridae* [2]. In young animals, the virus can cause fatal myocarditis. Even when an animal recovers from infection, long-term morbidity is common and up to 50% of recovered ruminants continue to harbor live viruses in the absence of clinical signs [3]. Based on the serological response to the capsid proteins, the FMD virus has been classified into seven serotypes denoted as O, A, C, SAT1, SAT2, SAT3 and Asia1. Each serotype has a different geographical range, with type O having the most worldwide spread [4].

The FMD viral genome and structural composition of the FMD viral capsid have been well documented; infectious FMD virus particles have non-enveloped icosahedral protein capsids containing a single-stranded, positive-sense RNA genome approximately 8500 nucleotides (nt) in length [5]. Genetic and antigenic analyses have classified the virus into seven serotypes; within each serotype, the strains were clustered into topo types and further lineages or genotypes according to their geographical origin [6]. There is no lasting cross-protection between serotypes and animals having an immune response against one serotype (either by vaccination or infection) will not be protected against the other serotypes [6].

Genetic lineages within these topotypes may also be limited in their polypeptide determines. The VP1 polypeptide determines virus attachment which helps in virus attachment to the host cell and its nucleotide sequence data are used for typing and subtyping of the FMD virus and in tracing the origin and transmission pathways of the disease [7]. Genetic and antigenic diversity is considered the highest for this serotype compared to the other Eurasian serotypes [8]. This variability may lead to the emergence of new strains of FMDV, making it difficult to prevent the disease through vaccination [9]. The genome contains a single open reading frame that codes for four structural (VP1-4) and eight to ten non-structural proteins. The viral capsid comprises 60 copies of each of the four different structural polypeptides, i.e., VP1, VP2, VP3 and VP4. The VP1 is the most variable of the capsid polypeptides and is considered to be highly immunogenic in nature [9]. One copy of each structural protein forms a protomer,

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five protomers form a pentamer and twelve pentamers form the complete capsid. VP1, 2 and 3 are on the surface of the virus and are comprised essentially of eight anti-parallel sheets linked to each other by loop structures to form a barrier, whereas VP4 is internal and has little secondary structure [10].

Complete genomic characterization of vaccine strains is vital in elucidating their genetic relationship with the circulating field strains, in detecting variation at the antigenic critical residues and in keeping track of any undesirable changes occurring in the vaccine strains' upscale culture propagation, which could compromise the overall antigenic and growth characteristics of the original seed virus [11]. The control of FMD relies on the early diagnosis of the virus in infected animals with the help of rapid and sensitive diagnostic techniques [12]. Foot-and-mouth disease viruses display high levels of genetic and antigenic variation. Vaccination is an effective way to control FMD; however, the protection conferred by vaccination or infection is usually serotype-specific and sometimes incomplete within a serotype [13]. It is therefore necessary to continuously monitor the FMD viruses circulating in the region and match them with the vaccine strains. The foot-and-mouth disease remains largely uncontrolled in the country because vaccination for prophylactic purposes is not being practiced except in a few dairy herds containing exotic animals. It is known to cause substantial economic losses, directly from the effect of the virus on animal health and indirectly through control efforts including quarantines and trade restrictions [14]. To initiate control measures for FMD, the following must be identified: the origin of infection, links between outbreaks, the extent of genetic variation of the causative viruses and the antigenic relationship of field isolates to the available vaccines.

The ability to control FMD is largely dependent on the availability of appropriate vaccines, which can be chosen based on epidemiological data, the phylogeny of the gene sequence for evolutionary analysis and the serological cross-reactivity of bovine post-vaccine serum with circulating viruses [15]. Furthermore, the determination of the antigenic and genetic profiles of FMDV strains is important for epidemiological studies and the selection of the most appropriate vaccine strains for a region where vaccination is practiced. For the purpose of epidemiological studies, molecular information on isolates at the regional level, including all neighboring countries, should make disease tracing more effective. As a result, the goal of this review is to learn how to diagnose and characterize FMDV field isolates at antigenic and genetic levels in order to match them to a vaccine strain.

## MATERIALS AND METHODS

**Virus Isolation:** According to the World Organization for Animal Health (OIE), Terrestrial Manual, virus isolation onto cell culture is considered the "gold standard" technique for FMD diagnosis [16]. FMDV diagnosis is based on clinical signs, followed by confirmation by laboratory tests. FMDV may be suspected in clinical material by the appearance of cytopathic effects (CPE) within 24 to 48hr. Following infection to BHK-21 cells [17]. The results may be confirmed by a virus neutralization test (VNT) assay and typing of the virus by ELISA [18]. This method is highly sensitive, but it is time-consuming, lasting between one and four days and it requires extraordinary laboratory facilities. The most sensitive cell culture to most FMD virus serotypes is the primary bovine thyroid, but these cells are difficult and exclusive and usually lose their susceptibility to FMDV after numerous passages [19].

**RNA Extraction:** Purification of viral RNA using the QIAamp Viral RNA Mini Kit can be fully automated on the QIAcube. The QIAcube performs the same steps as the manual procedure (lyse, bind, wash and elute), enabling us to continue using the QI Aamp Viral RNA Mini Kit for the purification of high-quality viral RNA. The QIAcube is preinstalled with protocols for the purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup [20]. RNA was extracted from the original samples and cell culture supernatants using the QIAamp Viral RNA Mini Kit (Qiagen, Crawley, West Sussex and the UK) following the manufacturer's recommendation. Reverse transcription (RT), polymerase chain reaction (PCR) to amplify the capsid-coding region, nucleotide sequencing and sequence analysis are carried out as described in Ullah *et al.* [8].

The presence of FMD viral genetic material is detected using real-time reverse transcription polymerase chain reaction (rRT-PCR). The rRT-PCR used to amplify genome fragments of FMD virus in diagnostic materials including epithelium, serum and probang samples [21]. RNA extraction is a technique for isolating and purifying RNA from in vivo tissues and samples. There are several methods for obtaining RNA. The presence of ribonucleases enzymes within the tissue cells complicates the extraction and purification process by quickly degrading the isolated RNA. Isolated and purified RNA can be used to detect gene expression, biomarkers, drug efficacy and much more. The currently favored technique for the detection of FMDV is the use of reverse

transcription-quantitative polymerase-chain-reaction (RT-qPCR) assays, which are rapid, sensitive and specific [22].

**Real-time Reverse Transcription-PCR:** Real-time reverse transcription-PCR (rRT-PCR) combined with an automated nucleic acid extraction process has been successfully used due to its high-throughput screening capacity, high accuracy and ease of data acquisition and handling [23]. The reverse transcription polymerase chain (RT-PCR) method is also useful for typing FMDV isolates based on sequencing but this method does not have optimal results in terms of specificity and sensitivity. Recently, real-time reverse transcription-polymerase chain reaction (rRT-PCR) assays have been developed with the capability of detecting FMDV in a variety of sample matrices [24].

**Antigenic Characterization of FMDV:** Antigenic characterization using serological tests like Virus Neutralization test, Enzyme linked Immunosorbent Assay VNT, ELISA and using defined sera or MAbs, are useful in showing antigenic diversity but they are unable to characterize strains individually and cannot be used to trace the origin of an outbreak. Antigenic characterization is used to compare field viruses with vaccine strains by determination of the serological relationship (r1value) using hyper-immune sera in ELISA or in VNTs using cell culture [5].Antigenic sites on the surface of the FMD virion have been identified for five of the seven serotypes of the virus (SAT1 and SAT3 being the only exception) [1]. Using VNTs, r1 values of ≥ 0.3 have been shown to reflect a close antigenic relationship between the field isolates and vaccine strains, indicative of good protection by the vaccine, whereas values < 0.3 reflect a more distant antigenic relationship, indicating that the vaccine is unlikely to protect against the field isolates. It has long been known that the main cell attachment site and the immune dominant region of FMDV are both located on a solvent-exposed region at the surface of the virion, namely in trypsin-sensitive areas of VP1 [25].

**Virus Neutralization Test:** The virus neutralization test (VNT) is currently considered as the "gold standard" for detection of antibodies to structural proteins of FMDV and is a prescribed test for import/export certification of animal products [26]. For antigenic analyses, the ability of each serum to neutralize each virus is assessed using a VNT. Vaccine matching is evaluated by comparison of the serum titter of a field strain to that of the vaccine strain against which the vaccine antiserum is prepared. The one-dimensional VNT technique suggests using one pre-liquated virus dose of 100TCID50 to neutralize anti-FMDV sera [27]. A VNT is carried out to determine the serum titter at a virus dose of 100TCID50 [13]. A 100 TCID50 is chosen because this is located at the linear part of the curve between the neutralization titter and the virus dose. This test is the gold standard for vaccine matching in FMDV and is preferred over LPBE and/ or CFT. This method has the advantage of being able to predict the neutralization titter at a fixed virus dose (100TCID50) and allows for greater flexibility in the virus titration that may occur due to cell variation for antigenic analyses; the ability of each serum to neutralize each virus is assessed using a VNT. The test is undertaken either using a two-dimensional or a one-dimensional VNT technique as recommended by the Office International des Epizooties [27].

**Liquid Phase Blocking ELISA:** Foot and mouth disease can also be diagnosed by demonstration of antibodies to a particular serotype using Liquid phase blocking ELISA (LPBE), particularly in FMD-free areas. The assay can also be used to measure protective antibody titers in vaccinated areas [7]. The principle of the LPBE assay is liquid-phase blocking of FMDV antigen by specific antibodies in the sera. The LPBE is performed according to the method of Bertram *et al*. [28]. The currently used LPB ELISA is the most effective tool available for assessing the immune response following vaccination or for evaluating vaccine efficacy [29]. LPB ELISA detects any residual antigen remaining after an overnight reaction between dilutions of serum and a pre-treated virus dose and is used in different FMD reference laboratories for vaccine matching [29]. This test has the advantage over virus neutralization tests in that the test is rapid (the result can be read in one day versus waiting three days in VNT) and uses smaller volumes of post-vaccination sera that are mostly available in the limited amount [29].

In the LPBE an r1-value of 0.4 is considered indicative of a good vaccine match; an r1-value between 0.2 and 0.4 showing significant differences from the vaccine strain but there may a good enough level of cross protection depending on the potency of the vaccine [29]. No significant difference is usually observed between the VNT and LPBE results [30]; however, there are occasional mismatches between the two sets of results which is probably because in the neutralizing assay the virus needs to be able to escape serum neutralization and replicate in cells and cause cytopathic effect, while the LPBE only measures binding of antibodies to immobilized

antigen. A LPBE is recognized by OIE as a suitable alternative for large-scale routine testing of seroconversion following FMD vaccination. The current method of LPBE, however, utilizes chemically inactivated FMD virus as the diagnostic antigen. The preparation of inactivated diagnostic FMDV antigen poses a risk, as it involves handling of live FMDV during the process [31].

**Genetic Characterization of FMDV:** The aligned, complete P1 nucleotide sequences are used to determine the most suitable nucleotide substitution model using j-Model Test and MEGA [15]. Variations in substitution rate among branches will be evaluated by comparing four different clocks in the basic local Alignment Search Tool BLAST. The maximum clade credibility (MCC) phylogenetic tree is inferred using the Bayesian Markov Chain Monte Carlo (MCMC) method. Then, a Bayes factor analysis in TRACER version1.5 is used to determine the best-fit mode l that resulted in the selection of an uncorrelated exponential relaxed molecular clock [32].

**Clustal Tool:** Clustal is aseries of widely used computer programs used in Bioinformatics for multiple sequence alignment. The first Clustal package featured a fast and simple method for making "guide trees." These are clustering's of the sequences that are used to decide the order of alignment during the later progressive alignment phase. The analysis of each tool and its algorithm is also detailed in the irrespective categories. All variations of the Clustal software align sequences using a heuristic that progressively build a multiple-sequence alignment from a series of pairwise alignments [33]. This method works by analyzing the sequences as a whole, then utilizingthe UPGMA/Neighbor- joining method to generate a distance matrix. Clustal Omega has the widest variety of operating systems out of all the Clustal tools. Clustal Omega is a package for making multiple sequence alignments. They have roughly fallen into two main groups: those that are fast and able to make very large alignments or those that are more accurate and restricted to smaller numbers of sequences [33].

**Nucleotide Sequencing:** Nucleotide sequencing and sequence analysis of Capsid VP1 is the most studied FMDV protein because of its significance for virus attachment and entry, protective immunity and serotype specificity [34]. This technique was first used for the study of the epidemiology of FMD by Beck and Strohmaier (1987). The sequences of the entire capsid coding region (P1) of the viruses are generated. RNA extraction from the cell culture grown viruses, reverse transcription (RT), polymerase chain reaction (PCR) to amplify the P1 region, sequencing, sequence analysis and assembling and alignment are performed as previously described. MEGA5 is used to determine nucleotide and aa variations [15]. The amplified capsid VP1 PCR products are either purified with a QIAquick PCR purification kit or a QIAquick gel extraction kit according to the manufacturer's instructions (Qiagen) [36]. RT-PCR primers are used for the direct sequencing of internal gene segments by using a BigDye terminator cycle sequencing kit and automatic DNA sequences. The Nucleotide sequence analysis has now become the definitive technique for the characterization of FMDV strains [10].

**Proteins Hydrophobicity Sequences:** Hydrophobicity is one of the primary forces driving the folding of proteins. Each of the 20 amino acids has a characteristic hydrophobicity a measure of the non-polarity of a molecule. On average, hydrophobic residues tend to be in the core of a protein, where solvent accessibility is low, whereas polar residues tend to reside on the surface, where solvent accessibility is high. When the average hydrophobic behavior of amino acids is generally true, one might expect that there should be a statistically significant correlation between the hydrophobicity sequence and the corresponding surface exposure pattern [37]. The hydrophobic interaction plays a central role in determining the overall fold of a protein quantifying the degree of variation between sequence and structure will be relevant to protein design based purely on hydrophobic polar patterning, in which the hydrophobicity sequence is assumed to dictate the final fold [38]. The most commonly used sequence alignment techniques possess three components: scoring scheme, gap model and alignment. The solubility and purity of a protein are two important aspects in biochemical and structural analyses of proteins, in which the expression of the protein is the first step [39].

**Basic Local Alignment Search Tool (BLAST):** A basic local alignment search tool (BLAST) is one of the most commonly used tools for comparing sequence information and retrieving sequences from databases and is thus an excellent starting point for teaching bioinformatics [39]. BLAST is used every day by thousands of biologists or bio-informaticians to scan genomic databases or to perform intensive sequence comparisons between large genomic datasets. It is a fundamental processing tool,

used in many bioinformatics pipelines [41]. There are several types of BLAST to compare all combinations of nucleotideorproteinBLASTisasequencesimilaritysearch program that can be used via a web interface or as a stand-alone tool to compare a user's query to a database of sequences queries with nucleotide or protein databases [42]. BLAST uses some heuristic methods to reduce the running time with little sacrifice in accuracy. BLAST performs comparisons between pairs of sequences, searching for regions of local similarity [43]. BLAST is used to identify the regions of local similarity between query sequence and database sequences by considering some threshold value.

**Basic Local Alignment Search Tool Nucleotide (BLASTn):** This program, given a DNA query, returns the most similar DNA sequences from the DNA database that the user specifies. Nucleotide BLAST refers to the use of a member of the BLAST suite of programs, such as BLASTn to search with a nucleotide "query" against a database nucleotide"of subject" sequences. There are two members of the BLAST suite of programs that are designed to make nucleotide-to-nucleotide alignments [40]. The first is the original BLAST nucleotide search program known as BLASTn. The BLASTn program is a general-purpose nucleotide search and alignment program that is sensitive and can be used to align transfer Ribonucleic acid (tRNA) or ribosomal Ribonucleic acid (rRNA) sequences as well as messenger Ribonucleic acid (mRNA) or genomic Deoxyribonucleic acid (DNA) sequences containing a mix of coding and noncoding regions. Mega BLAST is a more recently developed nucleotide-level BLAST program is about10 times faster than BLASTn but is designed to align sequences that are nearly identical, differing by only a few percent from one another [42].

**Basic Local Alignment Search Tool Protein (BLASTp):** This program, given a protein query, returns the most similar protein sequences from the protein database that the user specifies. BLASTp is Protein-to-protein sequence searches are performed using the original member of the BLAST suite of programs. The default word size for a BLASTp search is three; the default substitution matrix is the BLOSUM62 matrix. Changing the word size from three to two to increases the sensitivity of the search. Using a different substitution matrix can also have an effect on search sensitivity. During a "blastp" search, low-complexity regions of the query sequence are filtered to reduce the construction of spurious alignments and enhance search speed [40].

Standard protein-protein BLAST (blastp) is used for both identifying a query amino acid sequence and for finding similar sequences in protein databases. Like other BLAST programs, blastp is designed to find local regions of similarity. When sequence similarity spans the whole sequence, blastp will also report a global alignment, which is the preferred result for protein identification purposes [43].

## CONCLUSION AND RECOMMENDATION

Foot-and-mouth disease is the most transboundary severe disease characterized by short incubation periods compared to any other infectious diseases. Foot and mouth disease viruses display high levels of genetic and antigenic variation. Genetic lineages within these top types may also be limited in their geographical distribution. The VP1 is the most variable of the capsid polypeptides and is considered to be highly immunogenic in nature. Currently, there is no single vaccine used worldwide for the control of foot and mouth disease due to different vaccine strains in different geographical regions. Genetic and antigenic characterization of the major antigenic sites of FMDV is helpful for the development of specific diagnostic tests and protective vaccines. Vaccination is an effective way to control FMD; however, vaccine matching with the field strain is important to avoid vaccine failure. Thus, the serotype of the virus, the region of origin and any information on the characteristics of the virus may give indications of the vaccines most likely to provide an antigenic match. In addition, new variant viruses are emerging periodically and antigenic mismatch is one of the main reasons for vaccine failure, consequently, the vaccine strain requirement is different according to the serotypes and genotypes of viruses prevailing in or threatening different regions and vaccines have to be selected with care. This is mainly due to the lack of effective government strategy to control FMD through vaccination or movement of livestock control and the absence of systematic disease surveillance and reliable epidemiological data.

Based on the conclusions above, the following recommendations have been forwarded;

- It should produce vaccine matching which gives protection against the prevalent foot and mouth disease virus.
- There should be clear national policies and strategies set to prevent and control foot and mouth disease.

- Measures against FMD should aim at the control and, if possible, the eradication of the agent in the animal reservoir or zoo animals.
- The existing situation of foot and mouth disease in Ethiopia calls for coordinated nationwide epidemiological surveillance which is urgently required together with typing of infecting strains.

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