ISSN 2664-4010 (Print) & ISSN 2664-6730 (Online)
South Asian Research Journal of Agriculture and Fisheries
Abbreviated Key Title: South Asian Res J Agri Fish

| Volume-5 | Issue-4 | Jul-Aug -2023 | **DOI: 10.36346/sarjaf.2023.v05i04.002**

Review Article

Diagnostic Overview of Foot-And-Mouth Disease Viruse (FMDV)

Ephrem Shimelis[1*], Shimels Tesfaye[1], Fanos Tadesse[1]

[1]Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, NBH1, 4killo King George VI St, Addis Ababa, Ethiopia

***Corresponding Author:** Ephrem Shimelis
Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, NBH1, 4killo King George VI St, Addis Ababa, Ethiopia

Article History
Received: 14.12.2022
Accepted: 19.01.2023
Published: 24.08.2023

Abstract: Foot-and-mouth disease (FMD) is one of the highly contagious diseases of domestic animals. Sensitive, specific, and quick detection of FMD virus antigens and antibodies is an essential corner stone diagnostic tool at each tier of control strategy. Continued development of molecular and genetic technologies combined with novel analytical techniques will benefit many areas of FMD research and understanding. Genome sequencing provides excellent insight into the evolutionary and epidemiological dynamics of such viruses. The advent of next generation sequencing (NGS) technologies enables evolutionary studies of the entire viral swarm. Sequencing technologies have advanced uses of viral genomic data; which helps to understand the global distribution and Trans-boundary movements of Foot-and-mouth virus disease. Also sequencing information can be used to understand antigenic change within virus strains; therefore, the aim of this review is to assess various diagnostic techniques including conventional and advanced techniques both from agent and host response sides. Among the agent side virus isolation, RT-PCR, antigen ELISA and immune-chromatography strips are commonly used diagnostic tests. Whereas from the host side: infrared thermo gram, antibody ELISA and Virus neutralization are some mentions. Owing to current technological advancement: DNA sequencing and microarray, expressing a luciferase reporter and quantitative proteomics were reviewed. Therefore, potential confirmatory diagnostic test should be in combination with each other.

Keywords: Antigen, Antibody, Diagnostic test and FMDV.

1. INTRODUCTION

The 70% of Ethiopian society is depending on the livestock farming (Getachew, 2019). The estimated livestock population in Ethiopia indicated that 60.39 million heads of cattle, 31.30 million heads of sheep, 32.74 million heads of goats and 1.21 million heads of camel (COMESA, 2019, Barbara *et al*., 2019), however, the sector productivity were affected by infectious diseases (Gizaw *et al.*, 2020, Tegegne. 2020), such as: an economically important transboundary viral disease endemic of the Foot and mouth disease viruses (FMDV) (Ayelet *et al*., 2012, Mesfine *et al*., 2019). The FMDV imposes both the direct losses (low milk production, scarcity of draft power, retarded growth of young animals, abortion, long dry periods and myocarditis leads to young animal death and indirect losses (costs for quarantine enforcement, euthanizing and disposing of infected animals, vaccination, outbreak control, culling, compensating producers for destroyed animals and cleaning and disinfecting affected premises) (Jemberu *et al*., 2014, Tadesse, 2017, Hailu *et al*., 2017).

Foot-and-mouth disease virus (FMDV) is caused by single-stranded RNA virus of genus *Aphtho virus* genus within the *Picornaviridae* family that classified into seven serotypes (O, A, C, SAT1–3 and Asia 1) based on its immunologically and genetically variations (Gong *et a*l., 2020, Hosseini *et al*., 2016), due to lack of proofreading RNA dependent RNA polymerase that may control mutation rate (Yalew, 2018). The domestic livestock including cattle, pigs, sheep and goats are susceptible to the FMDV (Bhatta *et al*., 2012) in which the disease is characterized by high morbidity, fever, lameness and vesicular lesions on the mouth, tongue, feet, snout and teats of infected animals (Biswal *et al.*, 2012).

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Epidemiological surveys in Ethiopia indicated that FMD outbreaks reported every year from October to May. Current report shows that the number of FMD outbreak records in 2008 to 2018 was 4.33 times higher than outbreak reports in 1981 to 2007 (Wubshet *et al.*, 2019). According to the report of outbreak investigated at NAHDIC laboratory (2008-2019) by Gizaw et al. (2020), out of the samples submitted to the WRLFMD during 2008 to 2018, from 50% (258 out of 520) positive samples, Serotype O was detected every year, whereas serotypes A (2008-2009, 2015 and 2017-2019) and SAT 2 (2009-2010, 2014-2015 and 2018). The virus morbidity was 1.4 to 53.6% at animal level and up to 61% at herd level (Aman *et al.* 2020) with the mortality rate is 1–5% in adult animals and raise up to 20% in young stock due to acute myocarditis (Urge *et al.*, 2020).

The control of FMD relies on the early diagnosis of the virus in infected animals with the help of rapid and sensitive diagnostic technique (Ali *et al.*, 2018). Specific, rapid, sensitive and accurate diagnosis of FMDV followed by vaccination is an essential step in controlling of the disease (Farouk, 2006, Farag, 2019, Reid *et al.*, 2014). Isolation and identification to detect antigen and genome of the virus or serological evidence support the presence of the virus for diagnosis on clinical ground and to trace the disease spread (Chakraborty *et al.*, 2014). Continued development of molecular and genetic technologies combined with novel analytical techniques will benefit many areas of FMD research and understanding (Robinson *et al.,* 2014). The recent application of next-generation sequencing platforms to address important epidemiological and evolutionary challenges (Freimanis *et al*., 2016). Recent determination of the sequence of the foot and mouth disease virus genome has led to greater understanding of the structure and function of the virus genome, The development of proteomic technology has revolutionized our ability to assess protein changes on a global scale (Liu *et al*., 2011). Literature is important tools that inspire the people to adopt introduce and apply new technology including disease diagnosis in both field of medical and veterinary medicine; therefore, the objective of this review is to elaborate the current diagnostic approach of foot-and-mouth disease from the side of agent and hosts.

2. DIAGNOSTIC OVERVIEW OF FOOT-AND-MOUTH DISEASE VIRUSE (FMDV)

Due to the rapidity of spread of FMD and the serious economic consequences that can arise from an outbreak, prompt, sensitive and specific laboratory diagnosis and identification of the serotype of the viruses involved in disease outbreaks is essential (Jamal *et al.*, 2013). Production of effective pen side tests and increasing the usability and affordability of various FMD diagnostics for laboratories with limited resources would offer significant benefits to endemic regions in particular (Robinson *et al.*, 2014). NSP tests are not serotype specific, and can be used in both vaccinated and unvaccinated animal (Aftosa *et al.*, 2015).

2.1 Antigen diagnosis

2.1.1 Histopathology and immunohistochemistry

Immunohistochemistry (IHC) is one of antigen detection enable us to isolate FMDV from tissue sections (Mahmuda *et al.*, 2017). Tissues samples are immobile in 10% neutral phosphate buffered formalin and embedded in paraffin are stained by hematoxylin and eosin (H&E) for examination with inverted microscope (Yamada *et al*., 2018). Also, the Universal Immuno-enzyme Polymer method with a HISTFINE simple stain Max PO (M) kit (Nichirei, Tokyo, Japan) is used according to the manufacturer's instructions, labeled by a monoclonal antibody specific for FMDV serotype, counterstained with hematoxylin and its specificity is confirmed by monoclonal antibody immunofluorescence (IF) (Yamada *et al*., 2018). To conduct immune fluorescent stain, Cells are fixed in 4% of formaldehyde solution (40 minutes), washes with PBS and permeabilized in 0.1% triton (for 15 minutes), after a further PBS wash it block with buffer (PBS-BSA) (for 30 minutes), then primary antibody is added (wait for1hr) and cells are washed 5x with PBS and incubated with Alexa Fluor-conjugated secondary antibody for 45 minutes, finally cells are stained and imaged on the Spectra Max Mini Max 300 Imaging Cytometer and the total number of cell is calculated by using ToPro-3 fluorescence (excitation 625/20nm) while infected cells are counted using Alexa 488 fluorescence (excitation 460/20nm) (Logan *et al*., 2017).

2.1.2 Isolation and propagation of FMDV

FMD virus isolation is preferred Baby Hamster Kidney-21 (BHK-21) cells, however, the cell lines of baby hamster kidney (BHK-21) is less susceptible to specific animal-derived FMDV (Ibrahim *et al..* 2015). (Mahmoud et al., 2019). So hat the FMDV need the adaption of the virus to the first passaged of BHK21 adherent cells until cytopathogenic effect is develop rapidly (Dill *et al.*, 2018). The presence of the FMDV in the samples are appreciated on BHK-21 cell by the presence of CPE comprising rounding and flattening of the cells, breaking down of the intracellular bridges and finally cell death (Mahmud., 2018) in order to estimate the infection dose of the viruses the viral titers endpoint titration according to the Spearman-Kärber method and expressed as 50% tissue culture infectious dose (TCID50) per milliliter (Dill *et al*., 2018).

2.1.3 Reverse-transcription PCR (RT-PCR)

The reverse-transcription PCR (RT-PCR) can be used detect the FMDV by amplifying the FMDV genome fragment in diagnostic material by using universal l or serotype specific primers (Admassu *et al.,* 2015). The universal

reverse primer (BES-VP1R) used to amplify and detect the all FMDV without serotypes from the sample is retrieved from the conserved 2B region alignment of VP1 genomic sequences of serotype accessed from the Gen-Bank nucleotide database while the serotype-specific forward primers are retrieved from the hyper variable regions of the capsid coding gene (VP1/1D) (Sareyyüpoğlu *et al.*, 2017). The RT-PCR method is automated and allowing increased diagnostic throughout. The Real-time RT-PCR has several advantages over conventional RT-PCR where it is more rapid and sensitive with reduced amount of post-PCR processing steps and performed in a closed one-tube system and avoids potential cross contamination during sample preparation for post-PCR analysis and Monitoring PCR product formation in real time can be achieved through the use of dual-labeled hydrolysis (Taq Man) probes consisting of complementary sequences within the target (Subramaniam e*t al.*, 2012, El-shehawy *et al.*, 2012) (Robinson *et al.*, 2016).

2.1.4 Sandwich ELISA

ELISA typing is a qualitative assay for the identification of viral serotype by using indirect double antibody sandwich ELISA (Standards *et al.,* 2012). FMD antigen detection ELISA is shown to be rapid and simpler to perform, 100% specificity for heterologous FMDV and 80% sensitivity for detection of complete virus particles in clinical samples and large number of samples can be processed without risk of laboratory cross contamination (Sharma *et al.*, 2015).

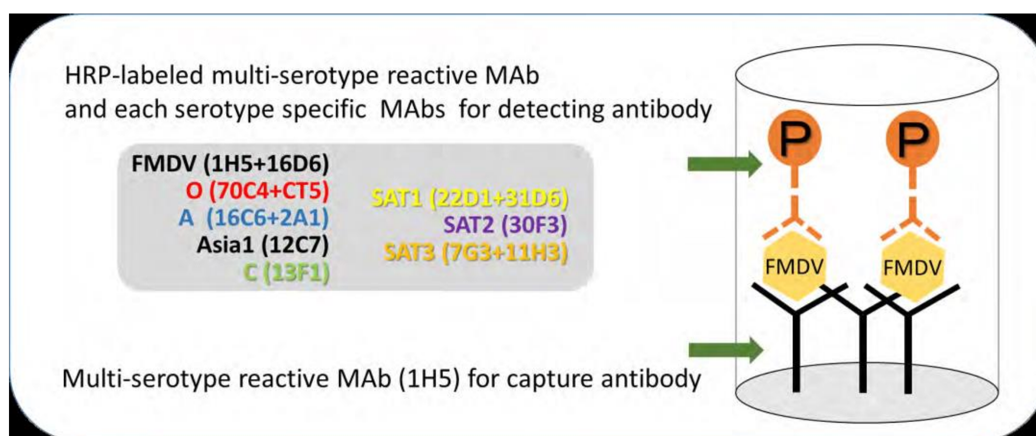

Fig 4: Newly developed ELISA for FMDV-antigen detection (Yamakawa *et al*., 2017)

2.1.5 Lateral flow immune chromatographic strip test (LFI)

Lateral flow device tests are one of the rapid tests that used for identification FMDV antigen, which can be performed in 15 minutes with specificity and sensitivity of (95% and 100%, respectively (Chitray *et al.*, 2020). The development of lateral flow strips for single serotypes is allowed in rapid detection of secondary outbreaks situation in which serotype is identified from initial out breaks and provide evidence of disease sprig around initial outbreaks (Yang *et al.*, 2013). LFIA have recently gained very high popularity in disease diagnosis especially in point-of care diagnosis (POC), low cost, easy to operate, stable over a wide range of weather conditions, have long shelf lives, require a small sample volume, and have a short reading time (Chalermthai *et al.*, 2017). Chromatographic strip based lateral flow assay (LFA) are highly sensitive, easy to use at animal side and can be carried out by unskilled personnel (Subramaniam *et al.*, 2012). A rapid lateral-flow assay (LFA) based on FMDV antigen detection, which is easy to use and can be utilized on the farm to reduce the time required for transport and laboratory diagnosis. FMDV (Oem *et al.*, 2009).

3.1.1. Infrared thermography detection of foot-and-mouth disease in the field

Thermal imaging has been considered for the early detection of clinical cases; however, a study reported that the assessment of hoof temperature by thermal imaging had limited accuracy as a means of detecting FMD (Robinson *et al.*, 2016). Animals with FMD often develop a fever with temperatures in excess of 40°C, which can be the first sign of the disease. In the absence of overt clinical signs, a pen-site rapid screening test that measures heat emission, such as IRT, could be essential in selecting potentially infected animals (Longjam *et al.*, 2011a). The information can be displayed in grey tones or as a colour scale. In a colour scale, the warmest areas are depicted as white or red, while the coolest areas appear blue or black. Combining thermography with other standard methods of lameness detection on-farm would improve the effectiveness and understanding of diagnostic competences of thermography (Alsaaod *et al.*, 2015).

2.1.6 Rapid detection of FMDV with optical microchip sensors

Functionalization of individual waveguide channels with target-selective antibodies confers localized sensitivity to specific biological agents; interactions between target antigens within the test sample and respective surface-immobilized antibodies are associated with localized changes in refractive index that results changes in sensor reflected

wavelength from specific sensing channels as it describes the use of Spectro SensTM optical microchip sensors (Bhatta *et al.*, 2012).

λ max = 2 Λ neff

Where λ max is the wavelength of light at which maximum reflectivity occurs, Λ defines the grating period and Neff is the average refractive index of the waveguide's composite structure. Hence, sensor reflected wavelengths are influenced by the local environment within the vicinity of specific gratings.

2.1.7 Microarray detection of FMDV

Microarray technology provides greater screening capabilities for FMDV detection and an attractive alternative to existing diagnostic methods (Subramaniam e*t al*, 2012). In general, the strategy of microarray hybridization is reversed to that of a standard dot-blot, leading to recurring confusion in the nomenclature (Schrenzel *et al*., 2009). A microarray-based test that used an FMD DNA chip containing 155 oligonucleotide probes, 35-45 base pairs (bp) long, and serotype-specific, designed from the VP3 and VP1-2A regions of the genome. A potential benefit of this technology is the possibility to use it on site in the form of a portable emerging format. Spectro Sens TM optical microchip portable biosensor system, functionalized with appropriate antibodies, was able to detect specific FMD viral particles very quickly (in about 10 min), easily and with a high level of accuracy (Niedbalski *et al*., 2016). DNA microarrays are becoming increasingly useful for the analysis of gene expression and single nucleotide polymorphisms (SNPs). A set of two forward primers and one reverse primer were also designed to allow amplification of approximately 1100 bp of target sequences from this region. The amplified target was labeled with Alexa-Fluor 546 dye and applied to the FMD DNA chip. A total of 23 different FMDV strains representing all seven serotypes were detected and typed by the FMD DNA chip (Longjam *et al*., 2011b).

3.1.2 DNA Sequencing and Phylogenetic structure of foot-and-mouth disease virus

Genome sequencing used to determine the evolutionary and epidemiological dynamics of such viruses (Towhid *et al.*, 2016). Next-generation sequencing is a new technology that show to generate sequence data directly from amplified single complementary DNA (cDNA) fragments. Furthermore, NGS enables sequencing on a high-throughput scale not afforded by previous Sanger based technologies, with a choice of several platforms being available to the end (Freimanis *et al*., 2016). Analyses have been performed on the complete P1 polyprotein, the genomic region encoding all four structural proteins that compose the viral capsid (1A, 1B, 1C and 1D). However, most of the work published regarding FMDV genomics is limited to the coding region of capsid protein 1D (also known as VP1). This information has been used to analyze variability, selective pressures and immunogenicity of FMDV. Phylogenetic analysis employing 1D sequences and a 15% nucleotide difference as a cut-off organizes FMDV strains into major groups or genotypes (Carrillo *et al*., 2014). Current efforts are focused upon the development of and requirement for a high-throughput capability, in order to assess the suitability of NGS to process and generate consensus-level whole genome sequences within an outbreak scenario. However, although significant progress has been made, the Comparative genomics of FMDV allows us to see how the virus mutates over time and locations and more importantly to trace the origin of the incurring FMDV strain. Genome sequencing provides excellent insight into the evolutionary and epidemiological dynamics of such viruses (Towhid *et al.,* 2016). Factors including sample-to-sample contamination, processing time and the development of simple analysis workflows, as well as the financial resources required to undertake these types of study, remain obstacles to be circumvented. Furthermore, with the increase in the use of NGS-based technologies with RNA viruses, such as FMDV, the development of analytical tools for such datasets, thereby maximizing their usefulness, will become an area of critical importance (Freimanis *et al*., 2016).

A sequence analysis based on multiple alignments for all sequences downloaded was used to examine how well the primers selected (probe sequences from the rRT-PCR selected matched at the target sequences. Multiple alignment among FMDV sequences and primer pairs was performed using the algorithm Clustal W method included in the BioEdit version 7.2.5 program (Rios *et al.*, 2018). Conventionally, the VP1 region (1D) sequence has been used for genetic characterization of foot-and-mouth disease virus (FMDV) strains because of its significance in virus attachment and entry, protective immunity and serotype specificity VP1-based phylogenetic analyses have been used widely to deduce evolutionary dynamics and the epidemiological relationship among the genetic lineages, and in tracing the authentic origin and movement of the outbreak strains (Mohapatra *et al.*, 2019). The phylogenetic analysis of the VP1 region was carried out with two methods both using the MEGA6.06 software package. In the neighbor joining method, the genetic distances between the nucleotide sequences were computed using the Tamura–Nei model (Tamura and Nei, 1993) and bootstrap values of the phylogenetic nodes were calculated out of 1000 replicates. In the maximum likelihood (ML) method, the Tamura–Nei model with uniform rates and the nearest-neighbor Interchange (NNI) with an initial tree obtained from NJ/BioNJ were selected (Ehizibolo *et al*., 2017).

2.2 Diagnosis of host responses (antibody) to pathogen (FMDV)

The other hand, the host cell activates a defense mechanism to limit viral growth to promote cell survival Interactions between the virus and its host determine the ultimate outcome of the battle for survival, which depends on

whether the virus is inhibited and gradually cleared by the host cells, enters a lytic infection, or becomes suppressed and enters persistent infection (Han *et al*., 2018). Several publications described the development of in-house tests for the detection of viral NSP-specific antibodies, the basis of most DIVA tests (differentiating infected from vaccinated animals. But regardless of the test used, relying on the presence of NSP antibodies to detect infection in vaccinated populations is imperfect, as vaccinated cattle may occasionally seroconvert, particularly after repeated vaccination (L. Robinson *et al*., 2016).

2.2.1 Virus neutralization test (VNT)

The virus neutralization test (VNT) is the "gold standard" technique for detection of antibodies to structural proteins of FMDV and is an approved test for the certification trade of animals and animal products, however, VNT is time- consuming, liable for contamination and requires special facilities in comparison to other serological tests that can use inactivated viruses as antigens (Mahmoud *et al*., 2019). It is sensitive and serotype specific, and plays an important role in FMDV diagnosis and vaccine matching. Nano-FMDV developing a rapid and high-throughput method to detect neutralizing antibodies against FMDV (Zhang *et al*., 2017). After 1 h, 150 µl l BHK-21cell suspension was added to each well, and the plates were incubated for 3day sat37°C and 5% CO_2, in a humidified atmosphere. After3 days, the plates were washed and the monolayers were stained with 50µl of amido black solution and the CPE was read macroscopically. The log10 titers of the virus with and without serum were calculated the neutralization index log10 titer was calculated by subtracting the virus log10 titer of the strain with test serum from the log10 titer of the strain without serum. All 3-w.p.v. sera were tested against FMDV serotype strains (Tekleghiorghis *et al.*, 2014).

2.2.2 Indirect sandwich ELISA for FMDV antigen detection

Indirect sandwich ELISA was used routinely for detection and serotyping of FMD virus in test samples. In last several years antigen ELISA is supplemented by real time RT-PCR for conformation of positive result (Polichronova *et al.,* 2010). Indirect ELISA for the detection of antibodies to the non-structural polypeptide (NSP) 3ABCof FMD virus in serum or plasma samples of large and small ruminant. The use of anti-3ABC specific monoclonal antibody (Mab) coated to the solid phase to trap the recombinant 3ABC polypeptide expressed in E coli micro titer plates are supplied pre-coated with the 3ABC antigen captured by Mab Appropriately diluted test sera are incubate with the trapped antigen, enabeling the specific antibodies eventually present in sample to bind to the 3ABC. An anti-ruminant IgG, peroxidase-conjugated Mab is dispensed: the anti-ruminant IgG bind to the FMD virus antibodies of the positive samples immune-complex with 3ABC.After incubation the unbound conjugate is removed by washing, and the TMB-chromogenic substrate is delivered into wells. A colorimetric reaction developer if conjugate has bound to sample antibody (Ateya *et al.,* 2017).

$$\text{Percentage positivity} = \frac{\text{net OD value of test sera}}{\text{net OD value of positive control serum}} \times 100$$

The ability to identify and selectively delete genes from a pathogen has allowed the development of "marker vaccines" that, combined with suitable diagnostic assays, allow differentiating infected from vaccinated animals (DIVA) by differentiation of antibody responses induced by the vaccine (no antibodies generated to deleted genes) from those induced during infection with the wild-type virus. A number of antigenic non-structural proteins (NSPs) of FMD were identified and out of which 3ABC gene appears to be the most reliable marker of FMD virus replication The deletion of NSP (3ABC) gene has been used for enabling DIVA approach for FMD (Cedivac-FMD inactivated vaccine) (Longjam *et al*., 2011b).

2.2.3 Solid-phase competitive ELISA (SPCE) for antibodies to FMDV

Commercial SPCE kit (IZSLER Brescia, Italy) was used for detection of serotype-specific antibodies to foot-and mouth disease virus according to the manufacturer's instructions. The criteria for the validity of the test are that the spectrophotometric readings must be ≥1 OD in the wells of the negative control while the positive control serum is expected to give ≥90% inhibition at 1/10 dilution and >50% inhibition at the second dilution (I/30). For screening purposes, the test sera is considered positive when it produces an inhibition ≥70% at the 1/10 dilution and negative when producing an inhibition of <70% at the dilution of1/10 (Ularamu *et al*., 2015). The Solid-phase competitive ELISA using selected neutralizing anti-FMD monoclonal antibody, specific for FMD serotypes (A, O, SAT2) is applied to measure antibodies against these serotypes. Percentage inhibition =100-(serum OD/reference OD*) x100. Reference OD=mean OD of four negative control wells =100%= 0% inhibition (Ateya *et al.*, 2017).

2.2.4 Quantitative Proteomic Analysis of Foot-and- Mouth Disease

Proteins were extracted from the organic TRIzol phase following the manufacturer's instructions. After a final washing step using95% ethanol, proteins were air-dried and the pellet was resuspended in freshly prepared 1% SDS (Carl Roth) by ultra-sonication. Quality and quantity of the isolated proteins were checked using a 12% polyacrylamide gel (SDS-PAGE) and a colorimetric bicinchoninic acid (BCA) assay (Pfaff *et al*., 2019). Beyond the identification of RNA-binding proteins by proteomic approaches, it can identify a previously unknown RNA binding capacity of the proteins (Fernandez *et al.*, 2019). Stable isotope labeling with amino acids in cell culture (SILAC) is used to quantify the host cell

gene expression profile as the expression of specific proteins would render differential effects on FMDV replication at different steps of the replication cycle, such as RNA replication, protein translation and virus titer(Guo et al., 2015). A dot-blot assay was developed to identify the binding of synthesized peptides to positive sera of FMDV type O (Yange *et al.,* 2017).

3.1.3. Allosteric biosensors

Allosteric biosensors allow detection of antibodies against different viruses by accommodating peptide sequences from surface viral proteins, acting as antibody receptors, into permissive sites of allosterically responsive recombinant β-galactosidases. These infection-specific FMDV biosensors can provide an effective and versatile alternative for the serological distinction of FMDV-infected animals (Longjam *et al*., 2011b). Recently, screening a phage displayed random 12-peptide library, it was found that positive phages displaying the consensus motif ETTXLE (X is any amino acid (aa)), which is highly homologous to 6ETTLLE11 at the N-terminus of the VP2 protein (structural protein) of the FMDV, a minimal epitopic region require to bind a monoclonal antibody of serotype independent FMDV (MAb 4B2) and thus can be used as a universal diagnostic candidate against(Longjam *et al*., 2011b).

2.2.5 Foot-and-mouth disease virus expressing a luciferase reporter

Recently Promega has developed a new luminescent reporter (smaller and brighter) by constructing a recombinant FMDV expressing reporter as a powerful bioluminescence technique in various fields of FMDV study (Zhang *et al.*, 2017). The bicistronic reporter plasmid TK-Renilla luciferase (Rluc). IRES-Fluc is constructed. cDNA encoding porcine G3BP1 was amplified by standard RT-PCR from total RNA extracted from porcine peripheral blood mononuclear cells and cloned into pCAGGS-Flag encoding a N-terminal Flag-tag. Mutagenesis of G3BP1 (S149A, Q280A, E284A, and E288A) was carried out by Overlap Extension PCR using specific mutagenic primers and validated by DNA sequencing (Wang *et al.,* 2018).

3. CONCLUSION AND RECOMMENDATION

Foot-and-mouth disease virus (FMDV) is an important veterinary pathogen which can cause widespread epidemics. Many works have been carried out to develop and validate diagnostic tests in regard to the FMD. By rapidly identifying potentially infected animals, sampling and testing could be done onsite, cutting the time of detection and allowing for faster implementation of quarantines in the control phase or quarantine release during the recovery phase of an FMDV. Antigenic and genotypic characterization of FMDVs is required for formulation of appropriate vaccine for effective control and eradication of the disease. In this review, antigenic, genetic, pathologic and advanced techniques involving the agent and the host were stated. These are expensive or inexpensive, portable and rapid as well as time consuming FMDV diagnostic techniques were overviewed. Therefor technique combinations have a great potential for the detection of FMDV to confirm the diagnosis in endemic areas.

Accordingly, the following recommendations are forwarded:

- For the emergence or re-emergence of any new lineage, the critically improved diagnostic tests to detect viral infection, effort need to be concentrated on the development of simple, rapid, noninvasive tests should be performed without expensive laboratory equipment.
- Potential validation of each diagnostic test could be in combination with other rapid pen-side diagnostic tests or conventional antigen detection methods.
- The polyclonal antibodies used in several assays should be replaced with the recombinant antibodies in the current assays.
- Immediate training and awareness should be given to all researchers, laboratory workers and others regarding individuals, in order to adopt, introduce and implement new diagnostic techniques.
- To confirm diagnosis accuracy, the tests should be done from both sides of pathogen detection and host responses characterizations.

LIST OF ABBREVIATIONS

BHK	Baby Hamster Kidney
BTY	bovine thyroid
CFT	Complement Fixation Test
CPE	Cytopathic Effect
DIVA	Differentiating infected from vaccinated animals
IRES	Internal ribosome entry site
FMD	Foot-And-Mouth Disease
gRAD	generic Rapid Assay Device
IHC	Immunohistochemistry
IF	immunofluorescence
IRT	Infrared thermography

LFT	Lateral flow immune chromatographic strip test
LK	lamb kidney
MEME	Mixed Effect Model of Evolution
Mab	Monoclonal Antibody
NGS	Next-generation sequencing
NIT	Neutralization Index Test
NSP	Nonstructural Protein
NAPPA	Nucleic Acid Programmable Protein Array
ORF	Open Reading Frame
PBS	Phosphate Buffered Saline
SAT	Southern African Territories
SPCE	Solid-phase competitive ELISA
TCID	Tissue Culture Infection Dose
VNT	Virus neutralization test
VP1	Viral Capsid protein one
WRL	World Reference Laboratory

Ethical Approval: No ethical clearance is needed for review.

Conflict of interest

The review was prepared by authors by revising of different papers by self; so there no one can cause issues of interest with this review.

Funding: The review was conducted without any fund expenditures.

Authors Contribution

The author was contributing in selection of the topics, reviewing different research papers and organizing in scientific ways for publications.

ACKNOWLEDGEMENTS

First, I praise and glorify my God, for his immeasurable help and blessing, letting me be here and successfully complete this piece of work. I feel a great pleasure to take this opportunity to express my deep and sincere gratitude to my seminar advisor Dr. Shimelis Tesfaye and Dr. Fanos Tadesse, who have helped and supported me starting from the selection of my review tittle to the final writing. Without their keen interest, kindness, unreserved advice, constant supervision, valuable guidance, constructive suggestions and encouragement, this review writing would not have been possible. My thanks also extend to Addis Ababa University College of veterinary medicine, which giving the chance and times of doing such like building and constructive review activities.

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