Recent progress in the diagnosis of foot-and-mouth disease: rapid field-based assays

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Abstract

The rapid and accurate diagnosis of foot-and-mouth disease (FMD) is critical for effective disease control. Most of laboratory-based methods can provide objective results within a few hours of sample receipt. However, the time taken to transport suspect material to the laboratory can be unacceptably long, often precluding laboratory confirmation in the event of an outbreak. Therefore, rapid and easy-to-perform tests, which can be used in the field (on-site) in case of a suspected disease outbreak, would be a valuable tool for veterinarians in the initial diagnosis of FMDV. The lateral flow immunochromatographic (LFI) test has been efficiently used for the detection of specific antibodies against FMDV non-structural proteins. FMDV serotypes O, A, Asia 1, SAT 2 and non-serotype-specific FMDV. Recently, several FMD-specific real-time RT-PCR (rRT-PCR) assays have been transferred to portable mobile platforms and evaluated for the detection of FMDV in the field: the Cepheid SmartCycler Real-time PCR machine, Enigma FL and LightCycler Nano System. The BioSeeq-Vet (Smiths Detection) was the first commercially available truly portable PCR instrument for the detection of viral RNA in the field. Additionally, a new portable amplification platform Genie I for the on-site detection of viral RNA by reverse-transcription loop-mediated isothermal amplification (RT-LAMP) has also been developed and evaluated. Infrared thermography (IRT), a quantitative method for the assessment of body surface temperature, may also be a useful tool for the early detection of FMDV in the field. In the absence of overt clinical signs, the IRT rapid screening test, which measures heat emission, can be essential in selecting potentially infected animals. Microarrays, a recently introduced technology for the laboratory diagnosis of FMD, offers greater screening capabilities for FMDV detection and can be regarded as an alternative to classical diagnostic methods.

Keywords: foot-and-mouth disease (FMD), on-site diagnosis, lateral flow device, portable real-time RT PCR platforms, infrared thermography, microarrays

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating trans-boundary viral disease that affects cloven-hoofed animals, such as cattle, sheep, goats, pigs and over 70 species of wildlife (17, 24). The causative agent, foot-and-mouth disease virus (FMDV), is a small positive-sense RNA virus belonging to the genus *Aphtovirus* of the *Picornaviridae* family (35). There are seven immunologically distinct serotypes of FMDV (A, O, C, Asia 1, SAT 1, SAT 2 and SAT 3) with a large number of variants spread over several regions in the world (17). Characteristic lesions of FMD include vesicles and epithelial erosions, as well as ulcers of the snout, tongue, hard and soft palate, teats, coronary band and feet. FMD causes significant economic losses in the affected countries due to costs associated with control and eradication measures, including the massive vaccination and/or destruction of infected herds, as well as decreased milk and meat production as a result of clinical disease (1). Furthermore, outbreaks of FMD result in sanitary barriers that prevent export of animals and animal by-products (23). At present, FMDV is widely distributed in different African and Asian countries, with serotype O having the highest prevalence, followed by serotype A (http://www.oie.int).

A rapid and accurate diagnosis of FMD is critical for effective disease control. In the event of an outbreak of FMD in a previously FMD-free country, diagnosis depends on an early recognition of the signs of the disease by the farmer and on rapid reporting to the relevant veterinary authorities so that the clinical symptoms can be evaluated. FMD cannot be distinguished clinically from other vesicular diseases, such as swine vesicular disease (SVD), vesicular exanthema of swine (VES) and vesicular stomatitis (VS). Consequently, laboratory tests are required for differential diagnosis. The ideal clinical specimens are fluid from unruptured vesicles and vesicular epithelium from ruptured lesions taken
from animals in the acute phase of the disease. Routine laboratory diagnosis of FMD can be performed by a combination of the enzyme-linked immunosorbent assay (ELISA) and virus isolation (VI), supplemented by reverse transcriptase PCR (RT-PCR) methods (32, 33). Moreover, the reverse-transcription loop-mediated isothermal amplification (RT-LAMP) has been developed to detect FMDV (11, 39). Most of these diagnostic methods, however, require the availability of a dedicated laboratory facility, highly trained personnel, stable reagents as well as multistep sample handling or preparation. In particular, VI requires a laboratory cell culture facility, is labour-intensive and time-consuming, requiring 4 to 6 days for test completion. The antigen capture ELISA is very rapid (taking up to 4 hours) and easy to perform, but the concentration of virus in the sample may be lower than the ELISA detection limit. Moreover, this test is suitable only for epithelium samples and does not have the analytical sensitivity required to confirm FMD-negative status. The conventional RT-PCR is labour-intensive, non-quantitative and subjective, which limits the number of samples that can be tested in a day. Real-time RT-PCR (rRT-PCR) is very sensitive and rapid, but can be expensive to perform because it requires a thermal cycler with a fluorescent detector and commercially available reagents (32, 33).

Most of laboratory-based methods can provide objective results within a few hours of sample receipt. However, the time taken to transport suspect material to the FMD laboratory can be unacceptably long, often precluding laboratory confirmation in the event of an outbreak. The period between reports of the disease and the collection and dispatch of samples to the laboratory can be protracted, which creates the possibility of sample contamination and degradation, so that virus may be very often not detected in the submitted samples. Besides, a long distance between the sample site collection and the laboratory can delay the diagnostic result, and consequently reduce the effectiveness of measures taken in case of an outbreak. These disadvantages have given rise to the idea that tests capable of being used on site or close to the outbreaks, without transferring samples to the laboratory, could play an important role in FMD control programmes. “Point of care” or “pen-site” diagnostic tests would open the possibility of a rapid, user-friendly, highly reliable identification of FMDV and economically feasible diagnosis of FMD under field conditions. Such tests would help in the selection of biological material that needs be sent to the laboratory for diagnostic serotyping and further characterization.

**The lateral flow immunochromatographic (LFI) test**

LFI tests have been widely used for the diagnosis of many contagious diseases and the detection of bioactive molecules, such as hormones, haptens and many others (47, 48). The lateral-flow colloidal-gold based immunoassay has been developed for the diagnosis of animals pathogens, such as porcine reproductive and respiratory syndrome virus (49), pathogenic isolates of *Vibrio harveyi* (38) and for the detection of antibodies to avian influenza virus strain H5N1 (10). Recently, the LFI test has been effectively used to detect specific antibodies against FMDV non-structural protein (44), FMDV serotypes O (46), A (21), Asia 1 (25) and all three FMDV serotypes O, A and Asia 1 (45). Moreover, the LFI test has also been developed for the detection of non-serotype-specific FMDV to support clinical diagnosis of FMD (13, 14, 31). This novel technology has several advantages over traditional immunoassays, including low cost, simplicity, rapid operations (results can be obtained within 15-20 min), no special equipment requirements, minimal personnel training and ease of use in the field or clinic. The detection of FMDV antigen by direct application of vesicular fluids and epithelial suspension from animals on an infected farm may reduce the possibility of diagnostic error arising from non-specific reactions. The diagnostic sensitivity of the LFI for FMDV was, at 84%, similar to 85%, obtained by the reference antigen ELISA, and the diagnostic specificity of the LFI was approximately 99%, compared to 99.9% for the reference ELISA (14). A limiting factor for non-serotype-specific strips was that they were not capable of identifying the serotype of FMDV, which reduced their usefulness in endemic countries, where a rapid identification of the serotype may be essential to disease control (37).

Svanodip FMDV-Ag, a lateral-flow device (LFD) for FMDV antigen detection, is commercially available from Boehringer Ingelheim Svanova (Sweden). This pen-site LFD based on 1F10 monoclonal antibody detecting all seven serotypes of the FMDV antigen in clinical samples can be used on the farm. The test captures antigen in swabs, fluid from unruptured vesicles/aphthae and vesicular epithelium from ruptured lesions. In this assay, FMDV-specific antibodies are bound to colloidal gold latex beads as well as to an immobiliser on the membrane (Fig. 1). If present in the clinical sample, FMDV antigen binds to the gold conjugate, forming an immune complex. The complex then migrates by capillary action along the membrane until it reaches the immobilised FMDV antibody. The complex will bind to the immobiliser antibody, resulting in an accumulation of colloid gold (a red/purple line – test line) in the test (T) window. A band in the test window indicates a positive result. The mouse anti-IgG antibody in the control window always binds colloid gold antibody, regardless of FMDV presence. The presence of this control (C) line ensures correct test performance (Fig. 1). The simplicity of the test procedure and the speed of obtaining an accurate result enables fast and informed decision making. The LFD is a valuable tool for the early detection of FMDV in the field, which is a great advantage in emergency situations, enabling the veterinary authorities to make an accurate diagno-
sis of FMD from clinical examination at the point of care. This LFD strip test for the detection of FMDV was presented and discussed by specialists from the Department of Foot-and-Mouth Disease at the Polish National Veterinary Research Institute during courses on vesicular diseases for the veterinary inspectorate. All participants had the possibility to perform the test by themselves, using a negative sample included in the kit. It was recommended to have this test in each veterinary inspectorate for pen-site diagnosis in case of a suspected FMD outbreak. This test can also be used in the laboratory for the initial diagnosis of samples from the field.

The field-portable real-time RT-PCR amplification assay

Currently, laboratory diagnosis of FMD depends mostly on the detection of viral RNA by RT-PCR assays (7, 32). To reduce the time from sampling to test results, which can be crucial when analyzing samples from an outbreak investigation or for the continuous monitoring of FMD, one option is to test the samples on the site of sampling. The ability to perform nucleic acid-based tests, such as PCR, in the field has proven to be a challenging goal largely because of the reliance on pre-processing of samples (nucleic acid extraction), which is a rate- and skill-limited step, given the relatively complex nature of RNA extraction methods (22). If portable RT-PCR platforms are to be used successfully in the field, ideally they should consist of a totally closed system capable of performing all steps of the assay: nucleic acid extraction, RT and PCR set-up, amplification and unambiguous interpretation of results. Until now, several FMD-specific rRT-PCR assays have been transferred to portable platforms and evaluated for diagnostic use (6, 19, 22, 27). A test developed by Callahan et al. (6) can be performed in 2 hours or less on a portable instrument, thus providing a rapid and sensitive method for the detection of viral RNA. The Cepheid SmartCycler Real-time PCR machine for the laboratory-based detection of FMD has been evaluated by the others (19). It was shown that the capacity of this instrument to detect RNA is greatly affected by the PCR reagents used for the assay. Used with PCR beads, it could not detect weakly positive samples, but when TaqMan core reagents were employed, its sensitivity was significantly increased. The Enigma FL portable instrument (Enigma Diagnostics, UK) can perform automated nucleic acid extraction followed by rRT-PCR and data analysis without the need for user intervention under laboratory or field conditions (42). The field version of this device is not yet commercially available, but it holds great promise, since the foot-and-mouth disease virus PCR detection assay has been successfully adapted to this platform (27). Some of the available models focus on user-friendlyness and robustness, e.g. Bio-Seeq (Smith Detection, UK) (34), while others are simple portable or compact thermocycles that are open to use with a virtually PCR protocol, e.g., LightCycler Nano System (Roche Diagnostic, Germany) (9). Portable real-time PCR platforms, such as Enigma FL and Bio-Seeq Plus, offer many advantages in endemic countries like Zambia (22). The Bio-Seeq Plus is a handheld user-friendly machine that relies on linear-after-the-exponential-polymerase chain reaction (LATE-PCR), which is an advanced form for asymmetrical PCR with end-point detection.

The BioSeeq-Vet was the first commercially available truly portable instrument for use in the field. It has been designed especially for field veterinarians. This diagnostic system comprises a portable briefcase-sized PCR instrument and a disposable sample preparation unit. Together, they offer rapid on-site identification within approximately 90 minutes, depending on the assay, under a wide range of weather conditions. It contains five combined PCR/sample preparation stations and allows the user to run the same assay on five samples or five different assays at the same time. The BioSeeq-Vet system is easily decontaminated by immersion and therefore allows the operator to analyse samples and obtain results from different sites in a single day. The battery-driven equipment is supplied with an integrated GPS, and the results can be sent immediately to the national server for the information of the veterinary authorities. This portable PCR instrument was successfully used during an FMD course in Turkey in 2009 attended by FMD

Fig. 1. Lateral-flow devices for FMDV detection. Modified, according to https://archives.eppo.int/Meetings/2009_conferences/diagnostic/18_King.pdf
specialists from all member countries of the European Union (UE), including a representative of the Polish National Veterinary Research Institute, Department of Foot-and-Mouth Disease, in Zduńska Wola. The BioSeeq Vet was recommended for use by the Polish veterinary inspectorates in the event of an outbreak (https://archives.eppo.int/Meetings/2009_conferences/diagnostic/18_King.pdf).

An alternative molecular detection technique is the RT-LAMP assay, which can be used for the detection of FMDV RNA under laboratory conditions (11, 39). Recently, a new portable amplification platform for pen-site detection of FMDV RNA by RT-LAMP has been developed and evaluated by OptiGene Ltd. (UK) in cooperation with the Pirbright Institute UK (http://www.optigene.co.uk/). This LAMP assay specific for 3D RNA polymerase-encoding region of the FMDV genome has been carried out on all extractions and run on the lightweight and robust Genie I portable instrument suitable for use in the field. It was concluded that the Genie I is a useful device for a rapid and reliable detection of FMDV with the LAMP assay and may potentially accelerate and improve the efficiency of FMDV diagnosis in the field.

Infrared thermography as a diagnostic tool for an early detection of foot-and-mouth disease in the field

Temperature is an important indicator for the diagnosis of animal diseases and for the evaluation of the physiological status of animals. Animals with FMD often develop a fever with temperatures in excess of 40°C, which can be the first sign of the disease. Vesicular lesions are associated with local inflammation, causing increased body temperature (1). Infrared thermography (IRT) is a rapid, non-invasive and quantitative method for the assessment of temperature, producing a pictorial representation of the distribution of the surface temperature of an object. This technique has been used over the recent years for the detection of animal diseases, including foot-and-mouth 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. Rainwater-Lovett et al. (30) studied a group of cattle experimentally infected with FMDV and measured temperatures as disease progressed. The maximum foot temperatures of healthy, directly inoculated, contact and vaccine trial cattle were measured by an IRT camera over the course of an FMD infection. An increase in temperature, sometimes prior to the development of visible lesions, was found (Fig. 2). The results indicate IRT as a promising screening technology for a quick selection of potentially infected animals for confirmatory diagnostic testing during FMD outbreaks. Thermograms of the mouth and feet of mule deer (Odocoileus hemionus) experimentally infected with FMDV revealed that only IRT pictures of the feet provided information useful in disease screening; foot temperatures increased significantly as lesions developed (12). This increase started 48 hours before any disease lesions were observed, indicating that disease detection before lesion development may be possible. Similar studies were performed at the Pirbright Institute on cattle, sheep and pigs infected with FMDV and then monitored for clinical signs and temperature for 4 days (2). The results revealed that pigs consistently showed the largest increases in the foot surface temperature after infection. Hot feet that could be directly attributable to FMDV infection were less likely in ruminants. The authors conclude that the practical value of IRT for the diagnosis of FMD is limited in ruminants because of variable temperatures observed prior to infection. In pigs, it may be more useful, and, in all cases, more measurements are needed, especially from uninfected animals. In the field, the IRT camera might be used to help identify animals (especially pigs) for further examination. In order to evaluate the temperature of normal, uninfected animals, IRT camera images of healthy cattle were taken over an extended period to determine hoof and eye temperatures and to examine how these vary with time and ambient conditions (15). It was found that the hoof temperature in animals varied from 10°C to 36°C and was primarily influenced by the ambient temperature and the animal’s activity prior to measurement. Eye temperatures were not affected by the ambient temperature, and they are a useful indicator of core body temperature. Eye temperature could be used to detect pyrexia as an indicator for selecting animals for closer examination. Owing to the variation in the hoof temperature in normal, healthy animals under various environmental conditions, the use of a single threshold hoof temperature will be a modest predictive
indicator of early FMDV infection, even if the ambient temperature is factored into the evaluation.

In summary, the IRT technique can be a useful tool for the early detection of FMD in the field. It is a rapid and remote technique. The ITR camera makes it possible to assess the body temperature of many animals in the herd at the same time from a long distance, e.g., from an airplane. Thus, instead of handling individual animals to examine them for lesions, IRT could be used to screen a herd or another group of animals for individuals with elevated foot temperatures. These could then be isolated for further testing and identification of the cause of the elevated body temperature. However, this preliminary diagnosis must be confirmed by FMD laboratory diagnostic methods.

Microarray technology and optical microchip sensors for detection of FMDV

DNA microarrays are becoming increasingly useful for the analysis of gene expression and single nucleotide polymorphism (18, 29). Microarray technology offers greater screening capabilities for pathogen detection and can be regarded as an alternative to classical diagnostic methods. Depending on the availability of appropriate probe sets, microarrays make it possible to identify several thousand microorganisms at the species, subtypes, or subtype level in a single assay. A number of diagnostic microarray chips have been developed to analyse multiple viral pathogens belonging to different virus families (43) to detect specific viruses (26, 40) or to define genetic variations undergone by viruses (5). Baxi et al. (3) developed 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 set of two forward primers and one reverse primer was also designed for the amplification of approximately 1100 bp of target sequences from these regions. The amplified target was labelled 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. To increase the overall robustness of the assay, each serotype was identified by several probes. It was shown that, in a single chip, this very rapid and sensitive microarray technology offers a unique capability to identify multiple pathogens. A potential benefit of this technology is the possibility to use it on site in the form of a portable emerging format (https://archives.eppo.int/Meetings/2009_conferences/diagnostic/18_King.pdf). Microarray technology was also used as a high-throughput method to analyse polymorphism within a short region of the FMDV genome encoding relevant functions in antigenicity and receptor recognition (28).

Over the last two decades, immune biosensors have become powerful and versatile detection tools for applications requiring on-site analysis, primarily because of their speed of analysis and simplicity of operation (20, 41). Stratophase Ltd. (Romsey, UK) has developed a transportable FMD detection unit using its optical microchip technology, which measures changes in the refractive index (RI) at the surface of an optical chip (http://www.biooptocsworld.com/). This SpectroSens™ 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 (4). The characteristics of the multiplexed detection platform highlight its potential for the in-field detection of FMD and prospective expansion into diagnosis of other infectious veterinary diseases.

Portable air sampling devices for use in the field

FMDV can be transmitted in a number of ways, including direct contact between infected and naive animals, indirect contact by mechanical transfer via people, wild animals and birds, vehicles, and animal products, e.g., milk or meat products, as well as by air (1, 16). The long-distance aerosol spread of FMDV occurs infrequently, as it requires particular climatic and epidemiological conditions (8). Until recently, the measurement of airborne FMDV in the field has not been attempted, and measurements have been restricted to the laboratory. At present, two portable non-invasive air samplers are available commercially: BioCapture 650 and BioBadge 100 (MesoSystems Technology, Inc., USA). These two rapid, handheld and simple-to-use devices successfully detected airborne virus in three proof-of-concept experiments involving pigs and cattle infected with FMDV (36). BioCapture and BioBadge air-samplers detected, respectively, 10.24 log_{10} and 11.23 log_{10} FMDV copies RNA/animal/24 hours after 5 minutes of collection near animals infected 3 days earlier by FMDV serotype Asia 1. Both devices have potential for use in the field, but for maximum benefit they will need to be integrated with a suitable portable viral RNA analysis instrument, e.g., Cepheid SmartCycler, Bio-Seeq Plus or Genie I machines. Currently, however, the sample fluid from both air-samplers must be analysed in the laboratory to detect FMDV, and further evaluation in the laboratory is required before any field measurements are considered.

References


