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A review of foot-and-mouth disease virus (FMDV) testing in livestock with an emphasis on the use of alternative diagnostic specimens

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Review

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Abstract

Foot-and-mouth disease virus (FMDV) remains an important pathogen of livestock more than 120 years after it was identified, with annual costs from production losses and vaccination estimated at €5.3–€17 billion (US$6.5–US$21 billion) in FMDV-endemic areas. Control and eradication are difficult because FMDV is highly contagious, genetically and antigenically diverse, infectious for a wide variety of species, able to establish subclinical carriers in ruminants, and widely geographically distributed. For early detection, sustained control, or eradication, sensitive and specific FMDV surveillance procedures compatible with high through-put testing platforms are required. At present, surveillance relies on the detection of FMDV-specific antibody or virus, most commonly in individual animal serum, vesicular fluid, or epithelial specimens. However, FMDV or antibody are also detectable in other body secretions and specimens, e.g., buccal and nasal secretions, respiratory exhalations (aerosols), mammary secretions, urine, feces, and environmental samples. These alternative specimens offer non-invasive diagnostic alternatives to individual animal sampling and the potential for more efficient, responsive, and cost-effective surveillance. Herein we review FMDV testing methods for contemporary and alternative diagnostic specimens and their application to FMDV surveillance in livestock (cattle, swine, sheep, and goats).

Introduction

Foot-and-mouth disease virus (FMDV) is a member of family *Picornaviridae*, genus *Aphthovirus* (Bachrach, 1977; Rodrigo and Dopazo, 1995; Rueckert, 1996). FMDV was the first virus of vertebrates to be identified, i.e., Loeffier and Frosch (1897) collected vesicular fluid, passed it through ceramic filters impermeable to bacteria, and reproduced clinical signs in cattle exposed to the filtrate. FMDV consists of a single-stranded, positive-sense RNA genome of approximately 8500 bases organized in three major regions (5′ non-coding regulatory region, polyprotein coding region, and 3′ non-coding regulatory region), with a polyadenylated 3′-end and a small, covalently linked protein (VPg) at the 5′-end. Polyproteins are post-translationally cleaved by viral protease into four structural proteins (VP1, VP2, VP3, and VP4) and eight non-structural proteins (NSPs; L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Ryan *et al.*, 1989). Structural proteins VP1, VP2, and VP3 assemble to form an icosahedral structure that is internally bound by VP4. NSPs function in virus replication and interactions with host cell factors and for processing of the structural proteins (Domingo *et al.*, 2002; Grubman and Baxt, 2004).

The classic clinical signs of FMDV infection (vesicles on the mouth and feet) were first described by Hieronymous Fracastorius (1546) after observing an outbreak in cattle near Verona, Italy (Mahy, 2005). FMDV is infectious for most animals in the order Artiodactyla (even-toed ungulates), but especially cattle, buffalo, swine, sheep, and goats (Burrows, 1968; Gibbs *et al.*, 1975*a*, 1975*b*; Bastos *et al.*, 2000; Kitching, 2002*a*, 2002*b*; Alexandersen and Mowat, 2005). In addition, more than 70 wildlife species are known to be susceptible to FMDV, including white-tailed deer (*Odocoileus virginianus*) (Snowdon, 1968; Fenner *et al.*, 1993; Moniwa *et al.*, 2012). FMDV in wildlife species is a serious concern because of the problems entailed in eradicating the virus from such populations. In the USA, 20,000 mule deer (*Odocoileus hermionus*) were killed in Stanislav National Forest to control the 1924–1926 FMDV outbreak in California.

The virus is highly contagious and, depending on the route of exposure, ≤10 tissue culture infectious doses are sufficient to infect and produce clinical disease in susceptible ruminants (Sellers *et al.*, 1971; Alexandersen *et al.*, 2003*b*). Although incubation time can be considerably longer, depending on dose and route of infection, viremia typically appears 24–48 h post-exposure with vesicles in the mouth and on the feet, thereafter (Yilma, 1980; Baxt and Mason, 1995). In an FMDV outbreak, transmission within and between populations can be

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rapid due to the short *in vivo* replication cycle (4–6 h) and acute onset of shedding (1–3 days) (Donaldson *et al*., 1987; Grubman and Baxt, 2004; Grau *et al*., 2015). The most common route of FMDV transmission is direct contact, however, transmission can occur over significant distances due to aerosol and mechanical dissemination of virus through water, feed, and fomites (Brooksby, 1982; Thomson *et al*., 2003). Clinically healthy FMDV carriers (reported up to 3.5 years in cattle, 9 months in sheep, and 4 months in goats) occur in both naïve and vaccinated ruminants, complicating control and eradication efforts (Pereira, 1981; Kitching, 1998; Alexandersen *et al*., 2002*a*, 2003*b*).

Infection elicits a rapid immune response, but as a result of extensive antigenic variation, immunity against one FMDV isolate does not necessarily protect against others (Bedson and Maitland, 1927; Galloway *et al*., 1948; van Bekkum *et al*., 1959; Gebauer *et al*., 1988; Salt, 1993; Sutmoller *et al*., 2003).Variation in VP1, VP2, and VP3 proteins made it possible for early investigators to use cross-neutralization tests to classify serotypes. In 1922, Vallée and Carré reported the presence of what is known today as serotype O in France and serotype A in Germany. Shortly thereafter, Waldmann and Trautwein (1926) reported what is now identified as serotype C in Germany (Brown, 2003). Three more serotypes (South African Territories; SAT 1, SAT 2, and SAT 3) were discovered in South Africa by Brooksby (1958) and Asia 1 was identified in Pakistan in 1957 (Brooksby and Rogers, 1957). Antigenic variation is a challenge to FMDV control because it has the potential to complicate vaccinology and diagnostics.

Depending on the geographic region, serotype-specific, inactivated FMDV vaccines are used to control clinical disease in endemic areas, but have also been used in FMDV eradication campaigns, e.g., Uruguay, Argentina, and Paraguay (Sumption *et al*., 2008). Outbreaks have occurred in every livestock-containing region of the world with the exception of New Zealand. According to the World Animal Health Organization (OIE, 2017), 66 countries are free of FMDV without vaccination, nine countries are free of FMDV with vaccination, and the remainder are endemically infected or lack reliable data upon which to base their true status.

Originally, FMDV used in vaccine production was derived from fluid collected from vesicular lesions on virus-inoculated cattle, just as was done previously for the production of smallpox vaccine virus (vaccinia virus) (Fenner, 1990; Sutmoller *et al*., 2003). Thus, Vallée *et al*. (1926) attempted to produce a FMDV vaccine using formaldehyde-inactivated fluid and loose epithelial tissues from vesicles on calves. Thereafter, Frenkel (1947) used macroscopic slices of tongue epithelium to propagate virus and prepare formaldehyde-inactivated vaccine. This approach was used by Rosenbusch *et al*. (1948) to produce enough FMDV vaccine to vaccinate more than two million cattle in Argentina (Brown, 2003). Over time, various cell lines, e.g., pig kidney (IBRS-2, MVPK-1), porcine kidney (LFBK), or baby hamster kidney fibroblast (BHK-21), were used in diagnostics or for FMDV propagation (Capstick *et al*., 1962; Snowdon, 1966; Swaney, 1976; Mohapatra *et al*., 2015). Among these cell lines, BHK-21 has been used for large-scale production of FMDV vaccine (Doel, 2003). In addition, a variety of contemporary vaccine technologies have been evaluated under experimental conditions, e.g., subunit, vector expression of subunit components, and DNA vaccines.

Protective immunity is directed toward structural proteins (Longjam *et al*., 2011). Therefore, elimination of NSPs (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) during vaccine production results in vaccinates without antibodies against these proteins, i.e., DIVA (differentiating infected from vaccinated animals) vaccines. That is, DIVA-vaccinated animals produce antibodies against FMDV structural proteins, but not against NSPs, whereas FMDV-infected animals produce antibodies against both structural and NSPs. Implementation of a DIVA strategy based on the detection of antibodies against NSPs in infected animals is used to monitor the ongoing success of FMDV eradication and to maintain 'FMD-free with vaccination' status (Bergmann *et al*., 2004). However, it has been observed that inadequately purified FMDV vaccines can contain enough residual NSP to induce anti-NSP antibody and produce false-positive enzyme-linked immunosorbent assay (ELISA) results (Uttenthal *et al*., 2010).

Whether the goal is early detection, sustained control, or eradication, diagnostically and analytically sensitive and specific (but affordable) FMDV surveillance tools are mandatory. Herein we review FMDV testing methods, contemporary and alternative diagnostic specimens, and their application in FMDV surveillance in livestock (cattle, swine, sheep, and goats).

Tests and testing

Prior to the development of the complement fixation test (1929), FMDV infection was diagnosed primarily by clinical signs, i.e., the presence of vesicles on epithelial surfaces of the feet, mouth, nasal regions, and mammary glands (Bachrach, 1968). However, diagnosis based on clinical signs is complicated by the fact that other viral infections, e.g., swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV), and vesicular exanthema of swine virus (VESV), may produce lesions which are indistinguishable from FMDV. Today, the detection of FMDV infections relies on the detection of FMDV-specific antibody (virus neutralization, antibody ELISA) or on the detection of the virus and/or viral components (virus isolation, antigen-capture ELISA, or reverse transcription-polymerase chain reaction (RT-PCR)). These techniques are reviewed below.

Virus detection

Direct complement fixation test

Prior to the development of techniques for virus isolation, Ciuca (1929) showed that the direct complement fixation test could be used to detect FMDV and serotype isolates. The method was based on the fact that guinea pig-derived complement is bound by virus–antibody complexes. If virus–antibody binding does not occur, the free complement will lyse sheep red blood cells (RBC) in the presence of anti-sheep RBC antibody. It was possible to identify FMDV serotypes using the direct complement fixation test because FMDV antibodies are serotype-specific. Later, Traub and Mohlmann (1943) used the direct complement fixation test to serotype FMDV in cattle. The direct complement fixation test is best used early in infection because it requires a high concentration of virus in the test specimen; thus, it is not useful when vesicles begin to resolve (Rice and Brooksby, 1953). Further, serum with pro- or anti-complementary activity will affect the test results (Ferris and Dawson, 1988).

Virus isolation

FMDV isolation was first described by Frenkel (1947) using primary bovine tongue epithelial cells, but Sellers (1955) and

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Bachrach *et al.* (1955) adapted primary bovine and swine kidney cells to FMDV diagnostics. Historically, bovine thyroid cells were considered the best primary cells for FMDV isolation, but more recently, continuous cell lines, e.g., IBRS-2, MVPK-1 clone 7, LFBK, BHK21, and BHK21-CT, have been widely used (Dinka *et al.*, 1977; Nair, 1987; House and House, 1989; Ferris *et al.*, 2006*a*, 2006*b*). Among several stable cell lines, bovine kidney cells expressing β6 and αV and integrin subunits (LFBK-αVβ6) were highly susceptible to all FMDV serotypes (LaRocco *et al.*, 2013). The availability of cell culture techniques and the realization that FMDV could be grown *in vitro* made typing of FMDV isolates more practicable (Rweyemamu *et al.*, 1982).

Virus isolation is the only way to confirm the presence of live FMDV, despite well-recognized challenges: (1) working with infectious FMDV presents a significant biosafety risk; (2) cell cultures lose susceptibility to the virus over time; (3) cell lines lose permissiveness to the virus over passages; (4) antibodies present in samples from infected animals may completely or partially neutralize FMDV; (5) virus isolation is much less analytically sensitive than RT-PCR (Alexandersen *et al.*, 2003*a*); (6) cytopathic effect can be caused by a variety of factors, not just FMDV, thus positive results must be confirmed using other methods.

Propagating virus on cell culture requires technical skill, adequate laboratory facilities, and more time than molecular assays. The diagnostic sensitivity of FMDV isolation varies among laboratories, virus serotype, and the cells used in the procedure (Alexandersen *et al.*, 2003*a*). Ferris *et al.* (2006*a*) evaluated test performance using a set of vesicular samples from FMDV-infected cattle (serotypes O, A, Asia 1, and SAT 2), SVDV-infected pigs, and negative control samples from cattle and pigs. Based on the results obtained from five European FMDV reference laboratories, bovine thyroid primary cells provided the highest rate of FMDV isolation (94%) when compared with primary lamb kidney cells (69%). The rate of isolation also varied among continuous cell lines: 69% for IBRS-2, 56% for BHK21 and 25% for BHK21-CT. In addition, primary bovine thyroid cells and IBRS-2 cells were susceptible to all FMDV serotypes, whereas primary lamb kidney cells, BHK21, and BHK21-CT cells were not susceptible to FMDV serotype SAT2. Data from more recent studies suggested that newer cell lines are highly susceptible to FMDV, but only partial comparisons among cell lines have been done. Brehm *et al.* (2009) compared primary bovine thyroid cells, IBRS-2, BHK21, and ZZ-R 127 (fetal goat) cell lines using FMDV isolates representing all seven serotypes. Although less sensitive than primary bovine thyroid cells, cell line ZZ-R 127 was more sensitive than the other cell lines included in the comparison. Similarly, LaRocco *et al.* (2013) found the LFBK-αVβ6 continuous cell line to be more susceptible to FMDV than primary lamb kidney, IBRS-2, and BHK21 cells.

Antigen-capture ELISA

The OIE (2012) recommends the use of FMDV antigen-capture ELISA for the detection of viral antigen and identification of viral serotype in clinical specimens and culture isolates (Roeder and Le, 1987; Ferris and Donaldson, 1992). Crowther and Abu-El Zein (1979) and Crowther and Elzein (1979, 1980) initially reported the use of antigen-capture ELISA to detect FMDV in cell culture and later applied the test to the detection of FMDV in cattle epithelial tissues. Currently, antigen-capture ELISAs based on polyclonal antibodies or various monoclonal antibodies targeting structural or NSPs are available (Hamblin *et al.*, 1984; Roeder and Le, 1987; Ferris and Dawson, 1988). Antigen-capture ELISA is capable of rapidly testing large numbers of samples, i.e., results can be obtained in 3–4 h (Alexandersen *et al.*, 2003*a*; Grubman and Baxt, 2004). However, the antigenic variability within and between serotypes further compromises the limited analytical sensitivity of the antigen-capture ELISA format. Studies showed that 70–80% of cell culture-positive samples and 63–71% of RT-PCR-positive oral/nasal swabs were detected by antigen-capture ELISA (Alexandersen *et al.*, 2003*a*; Morioka *et al.*, 2014).

Antigen-capture lateral-flow assay

FMDV antigen-capture lateral-flow assays or rapid chromatographic strip tests allow rapid on-site diagnosis in areas where the disease is endemic and in reference laboratories when a rapid result is needed. These assays detect FMDV antigens in vesicular fluids or epithelial suspension from infected animals using monoclonal or polyclonal antibodies (Reid *et al.*, 2001; Ferris *et al.*, 2009, 2010; Oem *et al.*, 2009; Jiang *et al.*, 2011). Oem *et al.* (2009) reported that a monoclonal antibody-based lateral-flow assay showed 87% diagnostic sensitivity and 99% diagnostic specificity for the detection of FMDV serotypes O, A, Asia1, and C when testing epithelial suspension specimens.

Reverse transcription-polymerase chain reaction

Relative to other virus detection methods, RT-PCR is considered to offer shorter turn-around time plus higher diagnostic and analytical sensitivity and specificity (Callens *et al.*, 1998; Reid *et al.*, 1998, 1999, 2000; Moss and Haas, 1999; Alexandersen *et al.*, 2003*a*; Shaw *et al.*, 2004; King *et al.*, 2006). Although FMDV is highly resistant to degradation in the environment, RT-PCR can detect nucleic acid from both infectious or inactivated virus, thereby reducing the impact of sample-handling deficiencies on virus detection (Cottral, 1969; Longjam *et al.*, 2011). The FMDV genome is heterogeneous. To avoid false-negative results, RT-PCR primers and probes must target nucleic acid sequences that are broadly conserved across all serotypes. For surveillance, RT-PCR can be used in parallel with virus isolation to achieve a more complete epidemiological picture (Laor *et al.*, 1992; Höfner *et al.*, 1993; Rodríguez *et al.*, 1994; Marquardt *et al.*, 1995; Callens *et al.*, 1998; Callens and De Clercq, 1999).

Realtime RT-PCR. Realtime RT-PCR (rRT-PCR) has been widely used in FMDV diagnosis because it offers improved analytical sensitivity and a simpler testing format, i.e., electrophoresis is not required. The first universal FMDV rRT-PCR used primers and probes specific to a highly conserved region within a polypeptide gene (P3) and achieved an analytical sensitivity for all FMDV serotypes estimated at 1×10^2 $TCID_{50}$ (Meyer *et al.*, 1991). Carrillo *et al.* (2005) compared whole-genome sequences of 113 FMDV isolates and found that the 5′UTR and 3D (RNA-dependent RNA polymerase gene) regions shared a high degree of nucleotide identity among FMDV isolates, i.e., 83% (5′UTR) and 91% (3D) homology. Further studies showed that primers and probes based on 5′UTR or 3D were analytically specific, i.e., no false positives were observed when testing specimens containing SVDV, VSV, or VESV (Callahan *et al.*, 2002; Reid *et al.*, 2002; Ferris *et al.*, 2006*a*, 2006*b*; Shaw *et al.*, 2007). Although OIE currently recommends the use of ‘universal’ primers and probes targeting conserved sequences within the 5′UTR or 3D regions, serotype-specific assays have also been created (Reid *et al.*, 2014; Bachanek-Bankowska *et al.*, 2016).

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Several studies have evaluated the diagnostic performance of 5′UTR and 3D FMD RT-PCRs. Using a variety of specimens containing viruses representing O, A, and Asia-1 serotypes plus serum and vesicular samples from FMDV-negative animals, Reid *et al.* (2014) reported no false-positive results and detection rates of 91 and 96% for 3D and 5′UTR rRT-PCRs, respectively.

Hindson *et al.* (2008) evaluated 5′UTR, 3D, or both rRT-PCRs using vesicular epithelium samples containing FMDV (serotypes O, C, Asia-1, SAT1, SAT2, SAT3), SVDV, or VESV. The diagnostic sensitivities of the 5′UTR and 3D rRT-PCRs were 87 and 97%, respectively. Combining the two methods resulted in a diagnostic sensitivity of 98%. King *et al.* (2006) compared the diagnostic sensitivities of the 5′UTR and 3D FMDV rRT-PCRs using 394 FMDV clinical specimens (serum, vesicular epithelium). Approximately 94% of samples (367 of 392) were positive on one of the two rRT-PCRs, with 88.1% (347 of 394) positive on both assays. Sequence analyses showed that all false-negative tests were the result of nucleotide substitutions within the region targeted by the primers or probes (King *et al.*, 2006). Therefore, laboratories may need to provide both 3D and 5′UTR RT-PCR testing, to reduce the likelihood of false-negative results caused by nucleotide changes in the 3D or 5′UTR target areas (Moniwa *et al.*, 2007).

Antibody detection

FMDV antibody detection methods are routinely used for several purposes; e.g., to certify animals or animal by-products are free from FMDV infection prior to import or export, to demonstrate previous exposure to FMDV or vaccination, or to evaluate antigenic matching of vaccines.

Indirect complement fixation test

The indirect complement fixation test was the first *in vitro* test developed for the detection of FMDV-specific antibody (Rice and Brooksby, 1953). The assay was further developed to detect FMDV antibodies from multiple FMDV serotypes (Nordberg and Schjerning-Thiesen, 1956; Sakaki *et al.*, 1977, 1978). At present, use of the indirect complement fixation test is only recommended by the OIE if FMDV ELISA testing is not available (OIE, 2012).

Serum-virus neutralization test

The FMDV serum-virus neutralization test (SVN) is a serotype-specific assay for the detection of neutralizing antibodies elicited by vaccination or infection (Golding *et al.*, 1976). Post-vaccination sero-surveys for FMDV are a major indicator in the assessment of preventive vaccination programs (Sobrino *et al.*, 2001). The existence of circulating neutralizing antibody is associated primarily with resolution of viremia (Pacheco *et al.*, 2010). The test may be performed on various cell lines, although Moonen and Schrijver (2000) found that BHK or IBRS-2 cells provided better results than PK-2 cells. The test is more specific than the indirect complement fixation test and is recommended for international trade by OIE, but the slow throughput (72 h to perform the test) is incompatible with rapid response and/or routine commerce. In addition, the assay's requirement for infectious virus mandates that testing be performed in a high-level biocontainment facility; often a difficult and expensive hurdle to clear.

Enzyme-linked immunosorbent assay

Elzein and Crowther (1978) developed the first indirect FMDV antibody ELISA. Subsequently, various FMDV ELISAs have been developed for the detection of antibodies and for serotyping of viruses (Rai and Lahiri, 1981; Ouldridge *et al.*, 1982; Hamblin *et al.*, 1984; Ouldridge *et al.*, 1984; Roeder and Le, 1987; Pattnaik and Venkataramanan, 1989). ELISAs are highly repeatable, cost-effective, and compatible with a variety of sample types, e.g., milk, probang, and oral fluid specimens (Burrows, 1968; de Leeuw *et al.*, 1978; Blackwell *et al.*, 1981; Longjam *et al.*, 2011; Senthilkumaran *et al.*, 2017).

Structural protein ELISAs. FMDV structural protein ELISAs are serotype-specific tests designed to detect antibodies elicited by vaccination or infection. Several blocking or competitive ELISAs have been developed based on serotype-specific polyclonal or monoclonal antibodies against capsid protein (VP1, VP2, and VP3), 146S particle, or 12S subunit epitopes (Cartwright *et al.*, 1980; Roeder and Le, 1987; Sáiz *et al.*, 1994). These assays provide faster throughput than SVN and avoid the need for tissue culture and live FMDV.

NSP ELISAs. Several FMDV-recombinant NSPs, e.g., 3ABC, 3AB, 3A, 3B, 3C, 2A, 2B, and 2C, have been used as target antigens in FMDV blocking and indirect ELISAs. Among these, antibodies against the 3ABC polyprotein are the most sensitive indicator of FMDV replication (Grubman, 2005; Henderson, 2005). Brocchi *et al.* (2006) compared four commercial NSP ELISAs and the OIE index screening assay using serum samples ($n = 3551$) from vaccinated and unvaccinated cattle, pigs, and sheep exposed to FMDV (Table 1). Diagnostic specificity was adequate for all tests (97–98%) and all tests displayed excellent diagnostic sensitivity (100%) when testing samples from recently exposed, unvaccinated animals. However, detection rates were much lower when testing vaccinated or exposed animals. As discussed previously, NSP antibody ELISAs can play a key role in verifying the status of countries considered FMD-free with vaccination.

Sampling and sample types

Serum

Transmission of FMDV can occur via respiratory, oral, or percutaneous exposure (Alexandersen *et al.*, 2003*a*). The initial replication of virus usually occurs at the site of entry followed by spread to regional lymph nodes through the circulatory system (Henderson and Brooksby, 1948). Viremia appears as soon as 24 h post-exposure (Cottral and Bachrach, 1968; Alexandersen *et al.*, 2002*a*, 2003*a*, 2003*b*; Kitching, 2002*a*; Murphy *et al.*, 2010). Viremia typically lasts 4–5 days in ruminants and 2–10 days in pigs, although the level of viremia is usually higher in pigs than in ruminants (Alexandersen *et al.*, 2001, 2002*b*, 2002*c*, 2003*a*, 2003*b*; Alexandersen and Donaldson, 2002; Hughes *et al.*, 2002; Murphy *et al.*, 2010; Stenfeldt *et al.*, 2016).

Serum specimens are useful for the detection of FMDV during viremia, i.e., serum samples collected ≤7 days post-infection (DPI) can be used for FMDV detection by virus isolation, rRT-PCR, and antigen-capture ELISA, with later samples useful for antibody detection. In cattle and pigs, Alexandersen *et al.* (2002*a*, 2002*b*, 2002*c*) reported the appearance of ELISA-detectable FMDV serum antibody by 5 DPI and neutralizing antibodies ≤2 days later (Alexandersen *et al.*, 2002*a*, 2003*a*). In sheep, ELISA-detectable serum antibody appeared by 9 DPI and neutralizing antibody between 6 and 10 DPI (Armstrong *et al.*, 2005). Coincident with the first detection of antibody is the progressive

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Table 1. Detection of FMDV infection in cattle using non-structural protein-based ELISAs (modified from Brocchi *et al.*, 2006)[a]

			Percent positive (95% confidence interval[b])				
	Days post-exposure	*n*	3ABC ELISA[c]	3ABC ELISA[d]	3ABC ELISA[e]	3ABC ELISA[f]	3B ELISA[g]
1. Non-vaccinated cattle exposed to infection (*n* = 54)	7–14	5	100 (48–100)	100 (48–100)	100 (48–100)	100 (48–100)	100 (48–100)
	15–27	27	100 (87–100)	100 (87–100)	100 (87–100)	100 (87–100)	100 (87–100)
	28–100	26	100 (87–100)	100 (87–100)	96 (80–100)	92 (75–100)	100 (87–100)
2. Vaccinated cattle exposed to infection (*n* = 285)	7–14	180–181	49 (41–56)	49 (41–56)	41 (34–49)	50 (43–58)	32 (26–40)
	15–27	131	60 (51–69)	53 (45–62)	50 (42–59)	53 (44–61)	38 (30–47)
	28–100	107–108	69 (60–78)	64 (54–73)	58 (49–68)	50 (40–61)	56 (46–65)
	>100	47	72 (57–84)	75 (60–86)	57 (42–72)	38 (25–54)	47 (32–62)

[a]Cattle serum samples obtained from experimental and known-status field animals.
[b]95% confidence intervals calculated from proportional data given in Brocchi *et al.* (2006).
[c]NCPanaftosa-screening (Panaftosa, Pan American Health Organization, Rio de Janeiro, Brazil).
[d]Ceditest® FMDV-NS (Cedi diagnostics B.V., Lelystad, The Netherlands. Currently produced and marketed as Priocheck® FMDV-NS by Thermo Fisher Scientific Prionics Lelystad B.v., Lelystad, The Netherlands).
[e]SVANOVIR™ FMDV 3ABC-Ab ELISA (Svanova, Upsala, Sweden).
[f]CHEKIT-FMD-3ABC (Bommeli Diagnostics/Idexx, Bern, Switzerland).
[g]UBI® FMDV NS ELISA (United Biomedical Inc., New York, USA).

clearance of virus from circulation and a reduction of virus in most tissues, with the exception of the pharyngeal region of ruminants (McCullough *et al.*, 1992; Alexandersen *et al.*, 2003*b*). Paired serum samples collected 7–14 days apart may be used to diagnose FMDV on the basis of rising antibody levels in response to infection. Serum antibody remains at high levels for several months post-infection and is detectable for years, with the exception that FMDV-specific antibody may be detected for only a few months in young pigs (Alexandersen *et al.*, 2003*a*). The use of filter papers for antibody detection or FTA cards for nucleic acid detection has been reported as a method to achieve diagnosis without the need to refrigerate or freeze serum samples (OIE, 2008).

Vesicular epithelium and fluid

During viremia, FMDV is distributed to secondary replication sites, i.e., tongue epithelium, nasal mucosa, salivary glands, coronary band epithelium, myocardium, kidney, spleen, and liver (Alexandersen *et al.*, 2001, 2003*a*). Viral amplification occurs mainly in cornified stratified squamous epithelium, e.g., feet, teats, dental pad, gum, tongue, and lips, resulting in the formation of liquid-filled vesicles (Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001; Arzt *et al.*, 2011*a*, 2011*b*). FMDV replication in pharyngeal epithelial and lymphoid tissues of cattle, sheep, and goats occurs in both the acute and persistent phases of disease (Alexandersen *et al.*, 2001, 2003*a*).

Depending on the route of introduction, vesicles become visible 1–3 days after exposure (Alexandersen *et al.*, 2001, 2003*a*; Murphy *et al.*, 2010; Arzt *et al.*, 2011*a*). However, subclinical infection is common in small ruminants, e.g., sheep and goats (Cardassis *et al.*, 1966; McVicar and Sutmoller, 1972; Gibson and Donaldson, 1986; Pay, 1988; Kitching, 2002*a*, 2002*b*). If present, vesicles are generally on the feet of small ruminants, e.g., sheep and goats (Cardassis *et al.*, 1966; Littlejohn, 1970; Gibson and Donaldson, 1986; Pay, 1988). If oral lesions are present in small ruminants, they commonly occur on the dental pad, rather than tongue as occurs in cattle (Geering, 1967). Vesicular fluid from unruptured vesicles on the dental pad, gum, tongue, lips, or feet of clinically affected animals is an ideal specimen for FMDV identification, because it contains a high concentration of virus (there are no reports of antibody detection in vesicular fluid) (Alexandersen *et al.*, 2001). However, vesicular fluid is generally only present in 1–2 days old lesions before they have ruptured. Alternatively, vesicular epithelium from ruptured lesions can be collected. FMDV can be detected in these samples up to 10–14 days (Alexandersen *et al.*, 2003*a*, 2003*b*). These samples should be stored in glycerine containing 0.04 M phosphate buffer saline (pH 7.6) (Ferris and Dawson, 1988). In the laboratory, the specimen can be crushed with sterile sand or beads and then mixed with laboratory medium to make a 10% suspension for testing by virus isolation, rRT-PCR, or antigen-capture ELISA (Oliver *et al.*, 1988; Reid *et al.*, 2001, 2002; Alexandersen and Donaldson, 2002; Sakamoto *et al.*, 2002). More recently, it has been reported that FMDV RNA can be detected directly from dry vesicular material by homogenizing the specimen with RNA extraction lysis buffer and then testing by rRT-PCR (Howson *et al.*, 2017, 2018). Collection of vesicular fluid and epithelium are most appropriate in the acute stage of infection. Both specimens are the sample of choice for FMDV detection using RT-PCR, antigen-capture ELISA, or antigen-lateral-flow device (OIE, 2017).

Buccal samples

FMDV replicates in pharyngeal epithelial tissues and may be detected in esophageal–oropharyngeal fluid by 24 h post-exposure (Salt, 1993). In ruminants, FMDV replication in pharyngeal epithelial tissues is protracted, i.e., the virus may be isolated from esophageal–oropharyngeal fluid samples for up to 9 months in sheep and 3.5 years in cattle (McVicar and Sutmoller, 1969; Straver *et al.*, 1970; Zhang and Kitching, 2001; Juleff *et al.*, 2008; Arzt *et al.*, 2011*a*, 2011*b*). In swine, infectious FMDV is present in most buccal samples for <28 days (oral fluid, nasal swab, esophageal–oropharyngeal fluid, tissues of the pharynx, tonsil, tongue, epiglottis, larynx, soft palate, nasopharynx, lung), although FMDV RNA was still detected in the tonsils of the soft palate at 28 DPI (Zhang and Bashiruddin, 2009; Arzt *et al.*, 2011*b*; Stenfeldt *et al.*, 2016).

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Probang sampling was first described as a method to collect esophageal–oropharyngeal fluid from ruminants by Sutmoller and Gaggero (1965). The sample is collected by inserting a small metal cup ('probang cup') on a long shaft through the mouth and into the pharyngeal region, thereby allowing the esophageal–oropharyngeal secretions to pool in the cup. Different sizes of probang cups are used, depending on the ruminant species. Probang sampling from pigs has only been reported under research conditions (Parida *et al.*, 2007; Stenfeldt *et al.*, 2013). Although esophageal–oropharyngeal fluid samples are the only method that offers a realistic chance of detecting FMDV in late-stage infection and in persistently infected ruminants, probang sampling is labor-intensive (involves several persons), requires technical skill, and necessitates animal restraint during the collection process (Kitching and Alexandersen, 2002; Kitching and Hughes, 2002; Kitching, 2002*a*, 2002*b*). Stenfeldt *et al.* (2013) reported that farmers were reluctant to allow probang sampling because of concerns that the collection process might harm their animals.

Oral fluid samples from pigs and cattle have been used to detect FMDV antibody and nucleic acid (Callens *et al.*, 1998; Alexandersen *et al.*, 2003*b*; Parida *et al.*, 2006, 2007; Stenfeldt *et al.*, 2013; Mouchantat *et al.*, 2014; Grau *et al.*, 2015; Vosloo *et al.*, 2015; Senthilkumaran *et al.*, 2017). Oral fluid samples can be collected from individual animals using various absorbent materials or from groups housed in the same space (pens or corrals) by allowing them to chew on rope suspended in the pen (Alexandersen *et al.*, 2003*b*; Prickett *et al.*, 2008; Kittawornrat *et al.*, 2010; Stenfeldt *et al.*, 2013; Mouchantat *et al.*, 2014; Vosloo *et al.*, 2015; Senthilkumaran *et al.*, 2017). Oral fluid collection is simple, non-invasive, rapid and cost-effective; for which reasons it has been widely applied to livestock surveillance, especially swine (Prickett and Zimmerman, 2010). FMDV can be detected in oral fluid samples by RT-PCR for up to 15 DPI in cattle, 8 DPI in sheep, and more than 27 DPI in pigs (Alexandersen *et al.*, 2003*b*; Parida *et al.*, 2007).

Conventional inactivated FMDV vaccines induce only a systemic antibody response whereas viral replication in infected animals produces both systemic and mucosal immune responses (McCullough *et al.*, 1992). Therefore, FMDV infection results in antibody-positive oral fluid or esophageal–oropharyngeal fluid samples, but vaccinated animals remain antibody-negative (DIVA) (Kitching, 2002*b*; Parida *et al.*, 2006). Virus neutralization assays and IgA-specific ELISAs for esophageal–oropharyngeal or oral fluid samples have been developed to detect FMDV-infected animals in vaccinated populations (Archetti *et al.*, 1995; Salt *et al.*, 1996; Amadori *et al.*, 2000; Parida *et al.*, 2006; Eblé *et al.*, 2007; Biswas *et al.*, 2008; Mohan *et al.*, 2008; Pacheco *et al.*, 2010; Stenfeldt *et al.*, 2016). Using an experimental ELISA based on a 3ABC polyprotein, FMDV-specific IgA was detected in oral fluids from pigs by 14 DPI (Senthilkumaran *et al.*, 2017). Earlier workers reported that FMDV-specific IgA could be detected in esophageal–oropharyngeal or oral fluid samples for up to 182 DPI in cattle and 112 DPI in pigs (Eblé *et al.*, 2007; Mohan *et al.*, 2008).

Mammary secretions

In 1968, Burrows reported that FMDV appeared in the milk of cattle exposed to infected animals an average of 2.2 days before clinical signs. Subsequent experiments showed extensive viral replication in bovine mammary gland parenchyma beginning 8–32 h post-exposure (Burrows *et al.*, 1971; Alexandersen *et al.*, 2003*b*). FMDV can also be detected in pig, sheep, and goat milk coincident with the appearance of viremia, but higher viral titers are present in sheep milk versus serum, suggesting either FMDV replication in small ruminant mammary gland tissue or the concentration of virus in milk (Burrows, 1968; McVicar, 1977; Arzt *et al.*, 2011*a*, 2011*b*). Blackwell *et al.* (1981) reported that FMDV could be shed in cattle mammary secretions for up to 14 DPI and was detectable in pasteurized whole milk, skim milk, cream, and cellular components in mammary secretions. Using rRT-PCR, FMDV nucleic acids can be detected in bovine milk for up to 23 days. These data justify the testing of bulk tank milk samples by RT-PCR for the early detection of FMDV in dairy herds (Reid *et al.*, 2006). Modeling the concentration of FMDV in bulk milk as a function of the number of cows shedding virus at any point in time, Thurmond and Perez (2006) predicted that FMDV nucleic acids could be detected in bulk tank milk samples between 2.5 and 6.5 days post-exposure, depending on the within-herd transmission rate. Further, it was predicted that nucleic acid could be detected in bulk tank milk before 10% of the cows showed clinical signs.

Individual and bulk tank milk samples have also been tested for FMDV-specific antibody, either for detection or for monitoring the response to vaccination (Armstrong and Mathew, 2001; Rémond *et al.*, 2002; Thurmond and Perez, 2006; Fayed *et al.*, 2013). Serum antibody is concentrated into mammary secretions by active transport mediated by neonatal Fc receptors on the basolateral surface of the mammary epithelial cells. As a result, mammary secretion collected from FMDV-infected cattle can contain higher levels of antibody than serum (Stone and DeLay, 1960). FMDV neutralizing antibody can be detected in mammary secretions within 7 days after exposure in cattle (Stone and Delay, 1960). ELISA-detectable FMDV antibody can be detected in mammary secretions for up to 12 months post-vaccination in cattle, 24 weeks post-vaccination in pigs, and 83 days post-vaccination in sheep (Burrows, 1968; de Leeuw *et al.*, 1978; Blackwell *et al.*, 1982; Francis and Black, 1983; Armstrong, 1997; Kim *et al.*, 2017).

Nasal and upper respiratory tract secretions

Respiratory tract mucosa is the initial site of FMDV replication and the virus is present in both upper and lower respiratory tract secretions during the acute phase of infection (Korn, 1957; Donaldson and Ferris, 1980; Alexandersen *et al.*, 2003*a*, 2003*b*). The specimens can be used in preclinical diagnosis because FMDV RNA may be detected in nasal swabs from 1 day before clinical signs through 10–14 days after the appearance of serum antibodies (Marquardt *et al.*, 1995; Callahan *et al.*, 2002; Alexandersen *et al.*, 2003*a*, 2003*b*). In pigs, FMDV RNA can be detected in nasal swabs from 6 h through 7 DPI, i.e., up to 2 days after the appearance of serum antibody (Alexandersen *et al.*, 2003*a*).

Aerosols

Airborne droplets or droplet nuclei containing infectious FMDV derived from secretions or excretions produced in respiratory, oral, and pedal epithelia present a significant challenge for prevention and control (Sutmoller and McVicar, 1976; Burrows *et al.*, 1981; Brown *et al.*, 1992; Sørensen *et al.*, 2000). Re-analysis of epidemiological and meteorological data collected

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Table 2. Temporal range for the detection of FMDV or viral components in alternative specimens

Species	Assay	Specimen	DPI[a]	References
Cattle	rRT-PCR	Serum	1–6	Alexandersen *et al.* (2003*a*, 2003*b*); Stenfeldt *et al.* (2013)
		Probang sample	1–553	Alexandersen *et al.* (2002*a*, 2002*b*, 2002*c*); Moonen *et al.* (2004); Subramanian *et al.* (2012); Stenfeldt *et al.* (2013)
		Buccal sample[b]	1–15	Alexandersen *et al.* (2003*a*, 2003*b*); Stenfeldt *et al.* (2013)
		Nasal swab	3–18	Subramanian *et al.* (2012)
		Feces	4–8	de Rueda *et al.* (2015)
	Virus isolation	Serum	1–8	Burrows (1968); Blackwell *et al.* (1982)
		Respiratory exhalation	1–4	Alexandersen *et al.* (2003*a*, 2003*b*)
		Probang sample	1–469	Burrows (1968); de Leeuw *et al.* (1978); Blackwell *et al.* (1982); Moonen *et al.* (2004); Subramanian *et al.* (2012)
		Nasal swab	3–5	Subramanian *et al.* (2012)
		Milk	1–13	Burrows (1968); de Leeuw *et al.* (1978); Blackwell *et al.* (1982)
Swine	Antigen-capture ELISA	Buccal sample[b]	1–7	Morioka *et al.* (2014); Senthilkumaran *et al.* (2017)
	rRT-PCR	Serum	1–11	Alexandersen *et al.* (2003*a*, 2003*b*); Doel *et al.* (2009); Stenfeldt *et al.* (2013); Senthilkumaran *et al.* (2017)
		Buccal sample[b]	1–27	Alexandersen *et al.* (2003*a*, 2003*b*); Parida *et al.* (2007); Stenfeldt *et al.* (2013); Mouchantat *et al.* (2014); Grau *et al.* (2015); Vosloo *et al.* (2015); Senthilkumaran *et al.* (2017)
		Respiratory exhalation	1–5	Parida *et al.* (2007); Doel *et al.* (2009)
		Pharyngeal swab	1–15	Mouchantat *et al.* (2014)
		Probang sample	1–27	Parida *et al.* (2007); Stenfeldt *et al.* (2013)
		Nasal swab	1–14	Alexandersen *et al.* (2003*a*, 2003*b*); Parida *et al.* (2007); Senthilkumaran *et al.* (2017)
		Feces	3–11	Fukai *et al.* (2015)
	Virus isolation	Serum	1–4	Alexandersen *et al.* (2003*a*, 2003*b*)
		Buccal sample[b]	1–5	Parida *et al.* (2007); Senthilkumaran *et al.* (2017)
		Respiratory exhalation	1–5	Alexandersen *et al.* (2003*a*, 2003*b*); Parida *et al.* (2007)
		Pharyngeal fluid	2–10	Burrows (1968)
		Nasal swab	2–5	Parida *et al.* (2007)
		Feces	3–4	Fukai *et al.* (2015)
		Rectal swab	1–7	Burrows (1968)

[a]Days post-inoculation (DPI) represent the minimum and maximum detection points reported.
[b]Buccal samples including samples collected with cotton swabs, cotton rope, or rope-in-a-bait collection devices.

during the 1982–1983 epidemic in Denmark suggested that FMDV was aerosolized and transmitted over a distance of 70 km (Christensen *et al.*, 2005). Infectious FMDV can be detected in respiratory exhalations 1–6 days post-exposure in cattle (Alexandersen *et al.*, 2003*a*). FMDV RNA can be detected in respiratory exhalations 6 h to 4 days post-exposure in pigs (Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001). Notably, pigs aerosolize more virus than ruminants, i.e., $1 \times 10^{6.1}$ median tissue culture infective dose ($TCID_{50}$) per day in pigs (Sellers *et al.*, 1971) compared with $1 \times 10^{4.3}$ $TCID_{50}$/day in cattle and sheep (McVicar and Sutmoller, 1976), because the virus replicates more extensively in swine respiratory mucosa (Oleksiewicz *et al.*, 2001; Alexandersen and Donaldson, 2002; Alexandersen *et al.*, 2002*a*, 2002*b*, 2002*c*; Arzt *et al.*, 2011*a*). In sheep, FMDV was detectable in respirations 17 h to 13 days post-exposure, i.e., FMDV is shed in aerosol 1–2 days before the appearance of clinical signs (Burrows, 1968; Sellers and Parker, 1969; Alexandersen *et al.*, 2002*b*). Experimentally, cattle and sheep can be infected by airborne exposure to as little as 1×10^1 $TCID_{50}$, whereas pigs require more than 1×10^3 $TCID_{50}$ (Alexandersen and Donaldson, 2002; Donaldson and Alexandersen, 2002; Alexandersen *et al.*, 2002*a*; Stenfeldt *et al.*, 2016).

Theoretically, on-farm air sampling could be used for preclinical non-invasive FMDV surveillance. For example, Pacheco *et al.* (2017) reported that FMDV RNA could be detected by passing air through filters, then disrupting the filters, extracting FMDV RNA, and performing RT-PCR. Similarly, Oem *et al.* (2005) detected FMDV RNA in exhaled air from infected cattle using a microchip-based hand-held air sampling device (Ilochip A/S, Denmark). FMDV RNA was harvested by washing the chip chamber with 25 µl of 0.1% (v/v) TritonX-100 solution (Sigma-Aldrich) followed by QIAamp Viral RNA Mini Kit (Qiagen, Germany) (Oem *et al.*, 2005). However, routine FMDV surveillance based on air sampling would need to account

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for the fact that viral aerosols are highly dynamic, non-uniform, and subject to atmospheric and climatic conditions (Verreault *et al.*, 2008). Furthermore, air sampling devices differ in recovery efficiency (Tseng and Li, 2005; Verreault *et al.*, 2008). Comparing all air sampling methods reported from 1960 to 2008, Verreault *et al.* (2008) concluded that no single sampling method was optimal for all climatic conditions. Perhaps for these reasons, aerosol sampling has primarily been a research tool for understanding and modeling the transmission of FMDV over distances.

Other sample types

Information concerning the shedding and detection of FMDV in urine or feces from FMDV-susceptible species is sparse, but shedding of FMDV in cattle urine and feces between 2 and 6 DPI has been reported (Bachrach, 1968; Garland, 1974). FMDV may be resistant in the environment, depending on the virus strain and the ambient conditions, and has been detected by virus isolation for up to 39 days in cattle urine and 14 days in feces (Bachrach, 1968; Cottral, 1969; Donaldson *et al.*, 1987; McColl *et al.*, 1995; Alexandersen *et al.*, 2003*a*). In general, urine and feces have not been considered suitable diagnostic specimens because they contain little virus and are likely to be mixed with environmental contaminants and other body fluids (Parker, 1971; Alexandersen *et al.*, 2003*a*). However, in the context of molecular diagnostics, these sample types may deserve further evaluation in terms of their suitability for environmental surveillance and monitoring.

Conclusions

FMDV remains an important pathogen of livestock more than 120 years after it was first identified because it is highly contagious, genetically and antigenically diverse, infectious for a wide variety of species, able to establish subclinically infected carriers in some species, and widely geographically distributed (Brito *et al.*, 2017). The 'burden of disease' imposed by FMDV is economically astonishing. Globally, Knight-Jones *et al.* (2017) estimated the annual costs from production losses and vaccination at €5.3–€17 billion (US$6.5–US$21 billion) in FMDV-endemic areas. In FMDV-free areas, they estimated the annual costs of FMDV outbreaks at ≥€1.2 billion (US$1.5 billion).

With good reason, the OIE and the Food and Agriculture Organization (FAO) have proposed the global eradication of FMD by the year 2030 (Rodriguez and Gay, 2011). This objective creates the need for alternative control methods, i.e., vaccines that provide broad-range protective immunity and diagnostic methods that can differentiate vaccinated from infected animals. Nevertheless, eradication is not feasible without the inclusion of accurate, cost-effective surveillance.

Historically, FMDV surveillance has typically been based on individual animal serum, vesicular fluid, or epithelial samples. Although current methods are still necessary for FMDV diagnoses, individual animal sampling and testing is impractical and expensive for surveillance in countries endemic with the disease. In an outbreak scenario, it would be feasible for individual sampling to occur. However, FMDV or antibody are also present in other body secretions, e.g., buccal and nasal secretions, respiratory exhalations (aerosols), mammary secretions, urine, feces, and environmental samples (Table 2). Alternative specimens can be used to support control and elimination programs by enabling herd-level sampling for FMDV surveillance at a lower cost and with less effort. Future research should focus on the development of diagnostic assays able to exploit the detection opportunities offered by alternative specimens, because, without these tools, the goal of FMDV eradication is unlikely to succeed.

References

Alexandersen S and Donaldson AI (2002) Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. *Epidemiology and Infection* **128**, 313–323.

Alexandersen S and Mowat N (2005) Foot-and-mouth disease: host range and pathogenesis. In Compans RW, Cooper MD, Honjo T, Melchers F, Olsnes S and Vogt PK (eds), *Foot-and-Mouth Disease Virus*. Berlin: Springer-Verlag Berlin Heidelberg, pp. 9–42.

Alexandersen S, Oleksiewicz MB and Donaldson AI (2001) The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT-PCR. *Journal of General Virology* **82**, 747–755.

Alexandersen S, Zhang Z and Donaldson AI (2002*a*) Aspects of the persistence of foot-and-mouth disease virus in animals – the carrier problem. *Microbes and Infection* **4**, 1099–1110.

Alexandersen S, Brotherhood I and Donaldson AI (2002*b*) Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O 1 lausanne. *Epidemiology and Infection* **128**, 301–312.

Alexandersen S, Zhang Z, Reid SM, Hutchings GH and Donaldson AI (2002*c*) Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. *Journal of General Virology* **83**, 1915–1923.

Alexandersen S, Zhang Z, Donaldson AI and Garland AJM (2003*a*) The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology* **129**, 1–36.

Alexandersen S, Quan M, Murphy C, Knight J and Zhang Z (2003*b*) Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *Journal of Comparative Pathology* **129**, 268–282.

Amadori M, Haas B, Moos A and Zerbini I (2000) IgA response of cattle to FMDV infection in probang and saliva samples. *EU FMD, Ras Gr, Borovets Appendix* **9**, 88–106.

Archetti IL, Amadori M, Donn A, Salt J and Lodetti E (1995) Detection of foot-and-mouth disease virus-infected cattle by assessment of antibody response in oropharyngeal fluids. *Journal of Clinical Microbiology* **33**, 79–84.

Armstrong RM (1997) The detection of antibodies against foot-and-mouth disease virus in sheep milk. *Journal of Virological Methods* **69**, 45–51.

Armstrong RM and Mathew ES (2001) Predicting herd protection against foot-and-mouth disease by testing individual and bulk tank milk samples. *Journal of Virological Methods* **97**, 87–99.

Armstrong RM, Cox SJ, Aggarwal N, Mackay DJ, Davies PR, Hamblin PA and Paton DJ (2005) Detection of antibody to the foot-and-mouth disease virus (FMDV) non-structural polyprotein 3ABC in sheep by ELISA. *Journal of Virological Methods* **125**, 153–163.

Arzt J, Baxt B, Grubman MJ, Jackson T, Juleff N, Rhyan J, Rieder E, Waters R and Rodriguez LL (2011*a*) The pathogenesis of foot-and-mouth disease II: viral pathways in swine small ruminants and wildlife; myotropism chronic syndromes and molecular virus–host interactions. *Transboundary and Emerging Diseases* **58**, 305–326.

Arzt J, Juleff N, Zhang Z and Rodriguez LL (2011*b*) The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. *Transboundary and Emerging Diseases* **58**, 291–304.

Bachanek-Bankowska K, Mero HR, Wadsworth J, Mioulet V, Sallu R, Belsham GJ, Kasanga CJ, Knowles NJ and King DP (2016) Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mouth disease virus serotypes circulating in East Africa. *Journal of Virological Methods* **237**, 114–120.

Bachrach HL (1968) Foot-and-mouth disease. *Annual Review of Microbiology* **22**, 201–244.

https://doi.org/10.1017/S1466252318000063 Published online by Cambridge University Press

Bachrach HL (1977). Foot and mouth disease virus: properties molecular biology and immunogenicity. In Diener TO and Romberger JA (eds), *Beltsville Symposia in Agricultural Research. I. Virology in Agriculture*, vol. **1**. New Jersey: Abacus Press, pp. 3–32.

Bachrach HL, Hess WR and Callis JJ (1955) Foot-and-mouth disease virus: its growth and cytopathogenicity in tissue culture. *Science* **122**, 1269–1270.

Bastos ADS, Boshoff CI, Keet DF, Bengis RG and Thomson GR (2000) Natural transmission of foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park South Africa. *Epidemiology and Infection* **124**, 591–598.

Baxt B and Mason PW (1995) Foot-and-mouth disease virus undergoes restricted replication in macrophage cell cultures following Fc receptor-mediated adsorption. *Virology* **207**, 503–509.

Bedson SP and Maitland HB (1927) Further observations on foot and mouth disease section D experiments on the cultivation of the virus of foot and mouth disease. *Journal of Comparative Pathology and Therapeutics* **40**, 79–93.

Bergmann IE, Malirat V, Neitzert E and Melo EC (2004) Vaccination: foot-and-mouth disease experience in South America. *Developments in Biologicals (Basel)* **119**, 273–282.

Biswas JK, Paton DJ, Taylor G and Parida S (2008) Detection of persistently foot-and-mouth disease infected cattle by salivary IgA test: the global control of FMD – Tools ideas and ideals. In Open session of the EU FMD Standing Technical Committee, Erice, Italy, 14–17 October 2008, pp. 377–382.

Blackwell JH, Wool S and Kosikowski FV (1981) Vesicular exocytosis of foot-and-mouth disease virus from mammary gland secretory epithelium of infected cows. *Journal of General Virology* **56**, 207–212.

Blackwell JH, McKercher PD, Kosikowski FV, Carmichael LE and Gorewit RC (1982) Concentration of foot-and-mouth disease virus in milk of cows infected under simulated field conditions. *Journal of Dairy Science* **65**, 1624–1631.

Brehm KE, Ferris NP, Lenk M, Riebe R and Haas B (2009) Highly sensitive fetal goat tongue cell line for detection and isolation of foot-and-mouth disease virus. *Journal of Clinical Microbiology* **47**, 3156–3160.

Brito BP, Rodriguez LL, Hammond JM, Pinto J and Perez AM (2017) Review of the global distribution of foot-and-mouth disease virus from 2007 to 2014. *Transboundary and Emerging Diseases* **64**, 316–332.

Brocchi E, Bergmann IE, Dekker A, Paton DJ, Sammin DJ, Greiner M, Grazioli S, De Simone F, Yadin H, Haas B and Bulut N (2006) Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* **24**, 6966–6979.

Brooksby J (1958) The virus of foot-and-mouth disease. In Smith K and Lauffer M (eds), *Advances in Virus Research*, vol. 5. Cambridge: Academic Press, pp. 1–37.

Brooksby JB (1982) Portraits of viruses: foot-and-mouth disease virus. *Intervirology* **18**, 1–23.

Brooksby JB and Rogers J (1957) Methods used in typing the virus of foot-and-mouth disease at Pirbright, 1950–1955. Methods of Typing and Cultivation of Foot-and-Mouth Disease Virus. Project 208 of OEEC, Paris, pp. 31–34.

Brown CC, Meyer RF, Olander HJ, House C and Mebus CA (1992) A pathogenesis study of foot-and-mouth disease in cattle, using *in situ* hybridization. *Canadian Journal of Veterinary Research* **56**, 189–193.

Brown F (2003) The history of research in foot-and-mouth disease. *Virus Research* **91**, 3–7.

Burrows R (1968) The persistence of foot-and-mouth disease virus in sheep. *Epidemiology and Infection* **66**, 633–640.

Burrows R, Mann JA, Greig A, Chapman WG and Goodridge D (1971) The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *Epidemiology and Infection* **69**, 307–321.

Burrows R, Mann JA, Garland AJM, Greig A and Goodridge D (1981) The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle. *Journal of Comparative Pathology* **91**, 599–609.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL and Rock DL (2002) Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. *Journal of the American Veterinary Medical Association* **220**, 1636–1642.

Callens M and De Clercq K (1999) Highly sensitive detection of swine vesicular disease virus based on a single tube RT-PCR system and DIG-ELISA detection. *Journal of Virological Methods* **77**, 87–99.

Callens M, De Clercq K, Gruia M and Danes M (1998) Detection of foot-and-mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot-and-mouth disease. *Veterinary Quarterly* **20** (Suppl. 2), 37–40.

Capstick PB, Telling RC, Chapman WG and Stewar DL (1962) Growth of a cloned strain of hamster kidney cells in suspended cultures and their susceptibility to the virus of foot-and-mouth disease. *Nature* **195**, 1163–1164.

Cardassis J, Pappous C, Brovas D, Strouratis P and Seimenis A (1966) Test of infectivity and dosage of foot and mouth disease vaccine in sheep. *Bulletin De L'office International Des Épizooties* **65**, 427–438.

Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A and Rock DL (2005) Comparative genomics of foot-and-mouth disease virus. *Journal of Virology* **79**, 6487–6504.

Cartwright B, Chapman WG and Brown F (1980) Serological and immunological relationships between the 146S and 12S particles of foot-and-mouth disease virus. *Journal of General Virology* **50**, 369–375.

Christensen LS, Normann P, Thykier-Nielsen S, Sørensen JH, de Stricker K and Rosenørn S (2005) Analysis of the epidemiological dynamics during the 1982–1983 epidemic of foot-and-mouth disease in Denmark based on molecular high-resolution strain identification. *Journal of General Virology* **86**, 2577–2584.

Ciuca A (1929) The reaction of complement fixation in foot-and-mouth disease as a means of identifying the different types of virus. *Epidemiology and Infection* **28**, 325–339.

Cottral GE (1969) Persistence of foot-and-mouth disease virus in animals, their products and the environment. *Bulletin De L'office International Des Épizooties* **70**, 549–568.

Cottral GE and Bachrach HL (1968) Foot-and-mouth disease viremia. *Proceedings of the Annual Meeting of the United States Livestock Sanitary Association* **67**, 463–472.

Crowther JR and Abu-El Zein EME (1979) Detection and quantification of foot and mouth disease virus by enzyme labelled immunosorbent assay techniques. *Journal of General Virology* **42**, 597–602.

Crowther JR and Elzein EA (1979) Application of the enzyme linked immunosorbent assay to the detection and identification of foot-and-mouth disease viruses. *Epidemiology and Infection* **83**, 513–519.

Crowther JR and Elzein EA (1980) Detection of antibodies against foot-and-mouth disease virus using purified *Staphylococcus* A protein conjugated with alkaline phosphatase. *Journal of Immunological Methods* **34**, 261–267.

De Leeuw PW, Van Bekkum JG and Tiessink JWA (1978) Excretion of foot-and-mouth disease virus in oesophageal-pharyngeal fluid and milk of cattle after intranasal infection. *Epidemiology and Infection* **81**, 415–426.

De Rueda CB, De Jong MC, Eblé PL and Dekker A (2015) Quantification of transmission of foot-and-mouth disease virus caused by an environment contaminated with secretions and excretions from infected calves. *Veterinary Research* **46**, 43. doi: 10.1186/ s13567-015-0156-5.

Dinka SK, Swaney LM and McVicar JW (1977) Selection of a stable clone of the MVPK-1 fetal porcine kidney cell for assays of foot-and-mouth disease virus. *Canadian Journal of Microbiology* **23**, 295–299.

Doel CMA, Gloster J and Valarcher JF (2009) Airborne transmission of foot-and-mouth disease in pigs: evaluation and optimisation of instrumentation and techniques. *The Veterinary Journal* **179**, 219–224.

Doel T (2003) FMD vaccines. *Virus Research* **91**, 81–99.

Domingo E, Baranowski E, Escarmís C and Sobrino F (2002) Foot-and-mouth disease virus. *Comparative Immunology Microbiology and Infectious Diseases* **25**, 297–308.

Donaldson AI and Alexandersen S (2002) Predicting the spread of foot and mouth disease by airborne virus. *Revue Scientifique et Technique-Office International Des Épizooties* **21**, 569–578.

Donaldson AI and Ferris NP (1980) Sites of release of airborne foot-and-mouth disease virus from infected pigs. *Research in Veterinary Science* **29**, 315–319.

https://doi.org/10.1017/S1466252318000063 Published online by Cambridge University Press

Donaldson AI, Gibson CF, Oliver R, Hamblin C and Kitching RP (1987) Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O1 and SAT 2 strains. *Research in Veterinary Science* **43**, 339–346.

Eblé PL, Bouma A, Weerdmeester K, Stegeman JA and Dekker A (2007) Serological and mucosal immune responses after vaccination and infection with FMDV in pigs. *Vaccine* **25**, 1043–1054.

Elzein EA and Crowther JR (1978) Enzyme-labelled immunosorbent assay techniques in foot-and-mouth disease virus research. *Epidemiology and Infection* **80**, 391–399.

Fayed AAA, Abdel-Halim MM and Shaker N (2013) Value of individual and bulk milk serology for surveillance and evaluation of vaccination programs used in dairy farms in Egypt to control FMD virus infection. *International Journal of Veterinary Medicine* **2013**, 1–11. doi: 10.5171/2013.730973.

Fenner F (1990) Poxviruses. In Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick J, Monath TP, and Roizman B (eds), *Virology*. New York: Raven Press, pp. 2113–2133.

Fenner FJ, Gibbs PJ, Murphy FA, Rott R, Studdert MJ and White DO (1993). Herpesviridae. In Fenner F, Bachmann PA and Gibbs PJ (eds), *Veterinary Virology*. London: Academic Press, pp. 337–368.

Ferris NP and Dawson M (1988) Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. *Veterinary Microbiology* **16**, 201–209.

Ferris NP and Donaldson AI (1992) The World Reference Laboratory for Foot and Mouth Disease: a review of thirty-three years of activity (1958–1991). *Revue Scientifique Et Technique-Office International Des Epizooties* **11**, 657–657.

Ferris NP, King DP, Reid SM, Hutchings GH, Shaw AE, Paton DJ, Goris N, Haas B, Hoffmann B, Brocchi E and Bugnetti M (2006*a*) Foot-and-mouth disease virus: a first inter-laboratory comparison trial to evaluate virus isolation and RT-PCR detection methods. *Veterinary Microbiology* **117**, 130–140.

Ferris NP, King DP, Reid SM, Shaw AE and Hutchings GH (2006*b*) Comparisons of original laboratory results and retrospective analysis by real-time reverse transcriptase-PCR of virological samples collected from confirmed cases of foot-and-mouth disease in the UK in 2001. *The Veterinary Record* **159**, 373–378.

Ferris NP, Nordengrahn A, Hutchings GH, Reid SM, King DP, Ebert K, Paton DJ, Kristersson T, Brocchi E, Grazioli S and Merza M (2009) Development and laboratory validation of a lateral flow device for the detection of foot-and-mouth disease virus in clinical samples. *Journal of Virological Methods* **155**, 10–17.

Ferris NP, Nordengrahn A, Hutchings GH, Paton DJ, Kristersson T, Brocchi E, Grazioli S and Merza M (2010) Development and laboratory validation of a lateral flow device for the detection of serotype SAT 2 foot-and-mouth disease viruses in clinical samples. *Journal of Virological Methods* **163**, 474–476.

Francis MJ and Black L (1983) Antibody response in pig nasal fluid and serum following foot-and-mouth disease infection or vaccination. *Epidemiology and Infection* **91**, 329–334.

Frenkel HS (1947) La culture du virus de la fièvre aphteuse sur l'épithelium de la langue des bovidés. *Bulletin De L'office International Des Épizooties* **28**, 155–162.

Fukai K, Yamada M, Morioka K, Ohashi S, Yoshida K, Kitano R, Yamazoe R and Kanno T (2015) Dose-dependent responses of pigs infected with foot-and-mouth disease virus O/JPN/2010 by the intranasal and intraoral routes. *Archives of Virology* **160**, 129–139.

Galloway IA, Henderson WM and Brooksby JB (1948) Strains of the virus of foot-and-mouth disease recovered from outbreaks in Mexico. *Proceedings of the Society for Experimental Biology and Medicine* **69**: 57–64.

Garland AJ (1974) *The Inhibitory Activity of Secretions in Cattle against Foot and Mouth Disease Virus* (Doctoral dissertation). School of Hygiene & Tropical Medicine, London. doi: 10.17037/PUBS.00878722.

Gebauer F, De La Torre JC, Gomes I, Mateu MG, Barahona H, Tiraboschi B, Bergmann I, De Mello PA and Domingo E (1988) Rapid selection of genetic and antigenic variants of foot-and-mouth disease virus during persistence in cattle. *Journal of Virology* **62**, 2041–2049.

Geering WA (1967) Foot-and-mouth disease in sheep. *Australian Veterinary Journal* **43**, 485–489.

Gibbs EPJ, Herniman KA, Lawman MJ and Sellers RF (1975*a*) Foot-and-mouth disease in British deer: transmission of virus to cattle sheep and deer. *The Veterinary Record* **96**, 558–563.

Gibbs EPJ, Herniman KAJ and Lawman MJP (1975*b*) Studies with foot-and-mouth disease virus in British deer (muntjac and sika): clinical disease recovery of virus and serological response. *Journal of Comparative Pathology* **85**, 361–366.

Gibson CF and Donaldson AI (1986) Exposure of sheep to natural aerosols of foot-and-mouth disease virus. *Research in Veterinary Science* **41**, 45–49.

Golding SM, Hedger RS and Talbot P (1976) Radial immuno-diffusion and serum-neutralisation techniques for the assay of antibodies to swine vesicular disease. *Research in Veterinary Science* **20**, 142–147.

Grau FR, Schroeder ME, Mulhern EL, McIntosh MT and Bounpheng MA (2015) Detection of African swine fever classical swine fever and foot-and-mouth disease viruses in swine oral fluids by multiplex reverse transcription real-time polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation* **27**, 140–149.

Grubman MJ (2005) Development of novel strategies to control foot-and-mouth disease: marker vaccines and antivirals. *Biologicals* **33**, 227–234.

Grubman MJ and Baxt B (2004) Foot-and-mouth disease. *Clinical Microbiology Reviews* **17**, 465–493.

Hamblin C, Armstrong RM and Hedger RS (1984) A rapid enzyme-linked immunosorbent assay for the detection of foot-and-mouth disease virus in epithelial tissues. *Veterinary Microbiology* **9**, 435–443.

Henderson LM (2005) Overview of marker vaccine and differential diagnostic test technology. *Biologicals* **33**, 203–209.

Henderson WM and Brooksby JB (1948) The survival of foot-and-mouth disease virus in meat and offal. *Epidemiology and Infection* **46**, 394–402.

Hindson BJ, Reid SM, Baker BR, Ebert K, Ferris NP, Tammero LFB and Hullinger PJ (2008) Diagnostic evaluation of multiplexed reverse transcription-PCR microsphere array assay for detection of foot-and-mouth and look-alike disease viruses. *Journal of Clinical Microbiology* **46**, 1081–1089.

Höfner MC, Carpenter WC and Donaldson AI (1993) Detection of foot-and-mouth disease virus RNA in clinical samples and cell culture isolates by amplification of the capsid coding region. *Journal of Virological Methods* **42**, 53–61.

House C and House JA (1989) Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: comparison of the sensitivity of cattle mice primary cell cultures cryopreserved cell cultures and established cell lines. *Veterinary Microbiology* **20**, 99–109.

Howson ELA, Armson B, Madi M, Kasanga CJ, Kandusi S, Sallu R, Chepkwony E, Siddle A, Martin P, Wood J and Mioulet V (2017) Evaluation of two lyophilized molecular assays to rapidly detect foot-and-mouth disease virus directly from clinical samples in field settings. *Transboundary and Emerging Diseases* **64**, 861–871.

Howson ELA, Armson B, Lyons NA, Chepkwony E, Kasanga CJ, Kandusi S, Ndusilo N, Yamazaki W, Gizaw D, Cleaveland S and Lembo T (2018) Direct detection and characterization of foot-and-mouth disease virus in East Africa using a field-ready real-time PCR platform. *Transboundary and Emerging Diseases* **65**, 221–231.

Hughes GJ, Mioulet V, Kitching RP, Woolhouse MEJ, Alexandersen S and Donaldson AI (2002) Foot-and-mouth disease virus infection of sheep: implications for diagnosis and control. *Veterinary Records* **150**, 724–727.

Jiang T, Liang Z, Ren W, Chen J, Zhi X, Qi G and Cai X (2011) Development and validation of a lateral flow immunoassay using colloidal gold for the identification of serotype-specific foot-and-mouth disease virus O A and Asia 1. *Journal of Virological Methods* **171**, 74–80.

Juleff N, Windsor M, Reid E, Seago J, Zhang Z, Monaghan P, Morrison IW and Charleston B (2008) Foot-and-mouth disease virus persists in the light zone of germinal centres. *PLoS ONE* **3**, e3434.

Kim AY, Tark D, Kim H, Kim JS, Lee JM, Kwon M, Bae S, Kim B and Ko YJ (2017) Determination of optimal age for single vaccination of growing pigs with foot-and-mouth disease bivalent vaccine in South Korea. *Journal of Veterinary Medical Science* **79**, 1822–1825.

https://doi.org/10.1017/S1466252318000063 Published online by Cambridge University Press

King DP, Ferris NP, Shaw AE, Reid SM, Hutchings GH, Giuffre AC and Beckham TR (2006) Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time reverse transcription-polymerase chain reaction assays. *Journal of Veterinary Diagnostic Investigation* **18**, 93–97.

Kitching RP (1998) A recent history of foot-and-mouth disease. *Journal of Comparative Pathology* **118**, 89–108.

Kitching RP (2002*a*) Clinical variation in foot and mouth disease: cattle. *Revue Scientifique et Technique-Office International Des Epizooties* **21**, 499–502.

Kitching RP (2002*b*) Identification of foot and mouth disease virus carrier and subclinically infected animals and differentiation from vaccinated animals. *Revue Scientifique Et Technique-Office International Des Epizooties* **21**, 531–535.

Kitching RP and Alexandersen S (2002) Clinical variation in foot and mouth disease: pigs. *Revue Scientifique Et Technique-Office International Des Epizooties* **21**, 513–516.

Kitching RP and Hughes GJ (2002) Clinical variation in foot and mouth disease: sheep and goats. *Revue Scientifique Et Technique-Office International Des Epizooties* **21**, 505–510.

Kittawornrat A, Prickett J, Chittick W, Wang C, Engle M, Johnson J, Patnayak D, Schwartz T, Whitney D, Olsen C, Schwartz K and Zimmerman J (2010) Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: will oral fluid replace serum for PRRSV surveillance? *Virus Research* **154**, 170–176.

Knight-Jones TJD, McLaws M and Rushton J (2017) Foot-and-mouth disease impact on smallholders – what do we know what don't we know and how can we find out more? *Transboundary and Emerging Diseases* **64**, 1079–1094.

Korn G (1957) Experimentelle untersuchungen zum virusnachweis im inkubationsstadium der maul-und klauenseuche und zu ihrer pathogenese. *Archiv Fur Experimentelle Veterinarmedizin* **11**, 637–649.

Laor O, Torgersen H, Yadin H and Becker Y (1992) Detection of FMDV RNA amplified by the polymerase chain reaction (PCR). *Journal of Virological Methods* **36**, 197–207.

LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B and Rodriguez LL (2013) A continuous bovine kidney cell line constitutively expressing bovine αvβ6 integrin has increased susceptibility to foot-and-mouth disease virus. *Journal of Clinical Microbiology* **51**, 1714–1720.

Littlejohn AI (1970) Foot and mouth disease in sheep. *The State Veterinary Journal* **25**, 3–12, 75–115.

Loeffier F and Frosch P (1897) Summarischer Bericht Ober die Ergebnisse der Untersuchungen der Kommission zur Erforschung der Maul-und Klauenseuche bei dem Institut for Infektionskrankheiten in Berlin Zentralblatt Fur Bakteriologie Mikrobiologie Und Hygiene. *Series A-Medical Microbiology Infectious Diseases Virology Parasitology* **1**, 257–259.

Longjam N, Deb R, Sarmah AK, Tayo T, Awachat VB and Saxena VK (2011) A brief review on diagnosis of foot-and-mouth disease of livestock: conventional to molecular tools. *Veterinary Medicine International* **2011**, 905768. doi: 10.4061/2011/905768.

Mahy BW (2005) Introduction and history of foot-and-mouth disease virus. In: Mahy BW (ed) Foot-and-Mouth Disease Virus. *Current Topics in Microbiology and Immunology* **288**: 1–8.

Marquardt O, Straub OC, Ahl R and Haas B (1995) Detection of foot-and-mouth disease virus in nasal swabs of asymptomatic cattle by RT-PCR within 24 h. *Journal of Virological Methods* **53**, 255–261.

McColl KA, Westbury HA, Kitching RP and Lewis VM (1995) The persistence of foot-and-mouth disease virus on wool. *Australian Veterinary Journal* **72**, 286–292.

McCullough KC, Pullen L and Parkinson D (1992) The immune response against foot-and-mouth disease virus: influence of the T lymphocyte growth factors IL-1 and IL-2 on the murine humoral response *in vivo*. *Immunology Letters* **31**, 41–46.

McVicar JW (1977) The pathobiology of foot and mouth disease in cattle: a review. *Bltn Centr Panam Fiebre Aftosa* **26**, 9–14.

McVicar JW and Sutmoller P (1969) The epizootiological importance of foot-and-mouth disease carriers. *Archives of Virology* **26**, 217–224.

McVicar JW and Sutmoller P (1972). Foot-and-mouth disease in sheep and goats: early virus growth in the pharynx and udder. *Proceedings of the Annual Meeting of the Unites States Livestock Sanitary Association* **73**: 400–406.

McVicar JW and Sutmoller P (1976) Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation. *Epidemiology and Infection* **76**, 467–481.

Meyer RF, Brown CC, House C, House JA and Molitor TW (1991) Rapid and sensitive detection of foot-and-mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene. *Journal of Virological Methods* **34**, 161–172.

Mohan MS, Gajendragad MR, Kishore S, Chockalingam AK, Suryanarayana VVS, Gopalakrishna S and Singh N (2008) Enhanced mucosal immune response in cattle persistently infected with foot-and-mouth disease virus. *Veterinary Immunology and Immunopathology* **125**, 337–343.

Mohapatra JK, Pandey LK, Rai DK, Das B, Rodriguez LL, Rout M, Subramaniam S, Sanyal A, Rieder E and Pattnaik B (2015) Cell culture adaptation mutations in foot-and-mouth disease virus serotype A capsid proteins: implications for receptor interactions. *Journal of General Virology* **96**, 553–564.

Moniwa M, Clavijo A, Li M, Collignon B and Kitching RP (2007) Performance of a foot-and-mouth disease virus reverse transcription-polymerase chain reaction with amplification controls between three real-time instruments. *Journal of Veterinary Diagnostic Investigation* **19**, 9–20.

Moniwa M, Embury-Hyatt C, Zhang Z, Hole K, Clavijo A, Copps J and Alexandersen S (2012) Experimental foot-and-mouth disease virus infection in white tailed deer. *Journal of Comparative Pathology* **147**, 330–342.

Moonen P and Schrijver R (2000) Carriers of foot-and-mouth disease virus: a review. *Veterinary Quarterly* **22**: 193–197.

Moonen P, Jacobs L, Crienen A and Dekker A (2004) Detection of carriers of foot-and-mouth disease virus among vaccinated cattle. *Veterinary Microbiology* **103**, 151–160.

Morioka K, Fukai K, Sakamoto K, Yoshida K and Kanno T (2014) Evaluation of monoclonal antibody-based sandwich direct ELISA (MSD-ELISA) for antigen detection of foot-and-mouth disease virus using clinical samples. *PLoS ONE* **9**, e94143.

Moss A and Haas B (1999) Comparison of the plaque test and reverse transcription nested PCR for the detection of FMDV in nasal swabs and probang samples. *Journal of Virological Methods* **80**, 59–67.

Mouchantat S, Haas B, Böhle W, Globig A, Lange E, Mettenleiter TC and Depner K (2014) Proof of principle: non-invasive sampling for early detection of foot-and-mouth disease virus infection in wild boar using a rope-in-a-bait sampling technique. *Veterinary Microbiology* **172**, 329–333.

Murphy C, Bashiruddin JB, Quan M, Zhang Z and Alexandersen S (2010) Foot-and-mouth disease viral loads in pigs in the early acute stage of disease. *The Veterinary Record* **166**, 10–14.

Nair SP (1987) Studies on the susceptibility and growth pattern of foot-and-mouth disease virus vaccine strains in two pig kidney cell lines. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **8**, 76–81.

Nordberg BK and Schjerning-Thiesen K (1956) Detection of complement fixing antibodies against foot-and-mouth disease in cattle serum. *The Journal of Infectious Diseases* **98**, 266–269.

Oem JK, Kye SJ, Lee KN, Kim YJ, Park JY, Park JH, Joo YS and Song HJ (2005) Development of a Lightcycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. *Journal of Veterinary Science* **6**, 207–212.

Oem JK, Ferris NP, Lee KN, Joo YS, Hyun BH and Park JH (2009) Simple and rapid lateral-flow assay for the detection of foot-and-mouth disease virus. *Clinical and Vaccine Immunology* **16**, 1660–1664.

OIE (World Organisation for Animal Health) (2008) *Manual of Standards for Diagnostic Tests and Vaccines*, 4th Edn. Paris: OIE, 957 pp.

OIE (World Organisation for Animal Health) (2012) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 7th Edn. Paris.

OIE (World Organisation for Animal Health) (2017) Resolution No 22: Recognition of the Foot and Mouth Disease Status of Member Countries 85th General Session of World Assembly May 2017.

https://doi.org/10.1017/S1466252318000063 Published online by Cambridge University Press

Oleksiewicz MB, Donaldson AI and Alexandersen S (2001) Development of a novel real-time RT-PCR assay for quantitation of foot-and-mouth disease virus in diverse porcine tissues. *Journal of Virological Methods* **92**, 23–35.

Oliver RE, Donaldson AI, Gibson CF, Roeder PL, Le PBS and Hamblin C (1988) Detection of foot-and-mouth disease antigen in bovine epithelial samples: comparison of sites of sample collection by an enzyme linked immunosorbent assay (ELISA) and complement fixation test. *Research in Veterinary Science* **44**, 315–319.

Ouldridge E, Barnett P and Rweyemamu MM (1982) Relative efficiency of two ELISA techniques for the titration of FMD antigen. *Veterinary Medicine and Animal Science* **22**, 142–151.

Ouldridge EJ, Barnett PV, Parry NR, Syred A, Head M and Rweyemamu MM (1984) Demonstration of neutralizing and non-neutralizing epitopes on the trypsin-sensitive site of foot-and-mouth disease virus. *Journal of General Virology* **65**, 203–207.

Pacheco JM, Arzt J and Rodriguez LL (2010) Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. *The Veterinary Journal* **183**, 46–53.

Pacheco JM, Brito B, Hartwig E, Smoliga GR, Perez A, Arzt J and Rodriguez LL (2017) Early detection of foot-and-mouth disease virus from infected cattle using a dry filter air sampling system. *Transboundary and Emerging Diseases* **64**, 564–573.

Parida S, Anderson J, Cox SJ, Barnett PV and Paton DJ (2006) Secretory IgA as an indicator of oro-pharyngeal foot-and-mouth disease virus replication and as a tool for post vaccination surveillance. *Vaccine* **24**, 1107–1116.

Parida S, Fleming L, Oh Y, Mahapatra M, Hamblin P, Gloster J, Doel C, Gubbins S and Paton DJ (2007) Reduction of foot-and-mouth disease (FMD) virus load in nasal excretions saliva and exhaled air of vaccinated pigs following direct contact challenge. *Vaccine* **25**, 7806–7817.

Parker J (1971) Presence and inactivation of foot-and-mouth disease virus in animal faeces. *Veterinary Record* **88**, 659–662.

Pattnaik B and Venkataramanan R (1989) Indirect enzyme-linked immunosorbent assay for the detection of foot-and-mouth-disease virus antigen. *Indian Journal of Animal Sciences* **59**, 317–322.

Pay TWF (1988) Foot and mouth disease in sheep and goats: a review. *Foot-and-mouth disease Bulletin* **26**, 2–13.

Pereira HG (1981) Foot-and-mouth disease virus. In Gibbs RPG (ed.), *Virus Diseases of Food Animals*, vol. **2**. New York: Academic Press, pp. 333–363.

Prickett JR and Zimmerman JJ (2010) The development of oral fluid-based diagnostics and applications in veterinary medicine. *Animal Health Research Reviews* **11**, 207–216.

Prickett J, Simer R, Christopher-Hennings J, Yoon KJ, Evans RB and Zimmerman JJ (2008) Detection of Porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. *Journal of Veterinary Diagnostic Investigation* **20**, 156–163.

Rai A and Lahiri DK (1981) A micro-enzyme-lavelled immunosorbent assay (MICORELISA) for the detection of foot-and-mouth disease virus antigen and antibody. *Acta Virologica* **25**, 49–52.

Reid SM, Forsyth MA, Hutchings GH and Ferris NP (1998) Comparison of reverse transcription polymerase chain reaction enzyme linked immunosorbent assay and virus isolation for the routine diagnosis of foot-and-mouth disease. *Journal of Virological Methods* **70**, 213–217.

Reid SM, Hutchings GH, Ferris NP and De Clercq K (1999) Diagnosis of foot-and-mouth disease by RT-PCR: evaluation of primers for serotypic characterisation of viral RNA in clinical samples. *Journal of Virological Methods* **83**, 113–123.

Reid SM, Ferris NP, Hutchings GH, Samuel AR and Knowles NJ (2000) Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. *Journal of Virological Methods* **89**, 167–176.

Reid SM, Ferris NP, Brüning A, Hutchings GH, Kowalska Z and Åkerblom L (2001) Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen. *Journal of Virological Methods* **96**, 189–202.

Reid SM, Ferris NP, Hutchings GH, Zhang Z, Belsham GJ and Alexandersen S (2002) Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. *Journal of Virological Methods* **105**, 67–80.

Reid SM, Parida S, King DP, Hutchings GH, Shaw AE, Ferris NP, Zhang Z, Hillerton JE and Paton DJ (2006) Utility of automated real-time RT-PCR for the detection of foot-and-mouth disease virus excreted in milk. *Veterinary Research* **37**, 121–132.

Reid SM, Mioulet V, Knowles NJ, Shirazi N, Belsham GJ and King DP (2014) Development of tailored real-time RT-PCR assays for the detection and differentiation of serotype O, A and Asia-1 foot-and-mouth disease virus lineages circulating in the Middle East. *Journal of Virological Methods* **207**, 146–153.

Rémond M, Kaiser C and Lebreton F (2002) Diagnosis and screening of foot-and-mouth disease. *Comparative Immunology Microbiology and Infectious Diseases* **25**, 309–320.

Rice CE and Brooksby JB (1953) Studies of the complement-fixation reaction in virus systems. V: In foot and mouth disease using direct and indirect methods. *The Journal of Immunology* **71**, 300–310.

Rodrigo MJ and Dopazo J (1995) Evolutionary analysis of the picornavirus family. *Journal of Molecular Evolution* **40**, 362–371.

Rodríguez A, Dopazo J, Saiz JC and Sobrino F (1994) Immunogenicity of non-structural proteins of foot-and-mouth disease virus: differences between infected and vaccinated swine. *Archives of Virology* **136**, 123–131.

Rodriguez LL and Gay CG (2011) Development of vaccines toward the global control and eradication of foot-and-mouth disease. *Expert Review of Vaccines* **10**, 377–387.

Roeder PL and Le PBS (1987) Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive rapid and reliable technique for primary diagnosis. *Research in Veterinary Science* **43**, 225–232.

Rosenbusch CT, Decamps A and Gelormini N (1948) Intradermal foot-and-mouth disease vaccine; results obtained from the first million head of cattle vaccinated. *Journal of the American Veterinary Medical Association* **112**, 45–47.

Rueckert RR (1996) Picornaviridae: the viruses and their replication. *Fields Virology* **1**, 609–654.

Rweyemamu MM, Pay TWF and Simms MJ (1982) The control of foot-and-mouth disease by vaccination. *Veterinary Annual* **22**, 63–80.

Ryan MD, Belsham GJ and King AM (1989) Specificity of enzyme-substrate interactions in foot-and-mouth disease virus polyprotein processing. *Virology* **173**, 35–45.

Sáiz JC, Cairó J, Medina M, Zuidema D, Abrams C, Belsham GJ, Domingo E and Vlak JM (1994) Unprocessed foot-and-mouth disease virus capsid precursor displays discontinuous epitopes involved in viral neutralization. *Journal of Virology* **68**, 4557–4564.

Sakaki K, Suphavilai P and Tokuda G (1977) Antibody estimation by indirect complement fixation test for foot-and-mouth disease in cattle. *National Institute of Animal Health Quarterly* **17**, 45–53.

Sakaki K, Suphavilai P and Chandarkeo T (1978) Inactivated-concentrated virus antigen for indirect complement fixation test of foot-and-mouth disease. *National Institute of Animal Health Quarterly* **18**, 128–134.

Sakamoto K, Kanno T, Yamakawa M, Yoshida K, Yamazoe R and Murakami Y (2002) Isolation of foot-and-mouth disease virus from Japanese black cattle in Miyazaki Prefecture Japan 2000. *Journal of Veterinary Medical Science* **64**, 91–94.

Salt JS (1993) The carrier state in foot and mouth disease – an immunological review. *British Veterinary Journal* **149**, 207–223.

Salt JS, Mulcahy G and Kitching RP (1996) Isotype-specific antibody responses to foot-and-mouth disease virus in sera and secretions of 'carrier' and 'non-carrier' cattle. *Epidemiology and Infection* **117**, 349–360.

Sellers RF (1955) Growth and titration of the viruses of foot-and-mouth disease and vesicular stomatitis in kidney monolayer tissue cultures. *Nature* **176**, 547–549.

Sellers RF and Parker J (1969) Airborne excretion of foot-and-mouth disease virus. *Epidemiology and Infection* **67**, 671–677.

Sellers RF, Herniman KA and Mann JA (1971) Transfer of foot-and-mouth disease virus in the nose of man from infected to non-infected animals. *Veterinary Record* **89**, 447–449.

Senthilkumaran C, Yang M, Bittner H, Ambagala A, Lung O, Zimmerman J, Giménez-Lirola LG and Nfon C (2017) Detection of genome antigen and

https://doi.org/10.1017/S1466252318000063 Published online by Cambridge University Press

antibodies in oral fluids from pigs infected with foot-and-mouth disease virus. *Canadian Journal of Veterinary Research* **81**, 82–90.

Shaw AE, Reid SM, King DP, Hutchings GH and Ferris NP (2004) Enhanced laboratory diagnosis of foot and mouth disease by real-time polymerase chain reaction. *Revue Scientifique et Technique* **23**, 1003–1009.

Shaw AE, Reid SM, Ebert K, Hutchings GH, Ferris NP and King DP (2007) Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease. *Journal of Virological Methods* **143**, 81–85.

Snowdon WA (1966) Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature* **210**, 1079.

Snowdon WA (1968) The susceptibility of some Australian fauna to infection with foot-and-mouth disease virus. *The Australian Journal of Experimental Biology and Medical Science* **46**, 667–687.

Sobrino F, Sáiz M, Jiménez-Clavero MA, Núñez JI, Rosas MF, Baranowski E and Ley V (2001) Foot-and-mouth disease virus: a long known virus but a current threat. *Veterinary Research* **32**, 1–30.

Sørensen JH, Mackay DKJ, Jensen CØ and Donaldson AI (2000) An integrated model to predict the atmospheric spread of foot-and-mouth disease virus. *Epidemiology and Infection* **124**, 577–590.

Stenfeldt C, Lohse L and Belsham GJ (2013) The comparative utility of oral swabs and probang samples for detection of foot-and-mouth disease virus infection in cattle and pigs. *Veterinary Microbiology* **162**, 330–337.

Stenfeldt C, Pacheco JM, Smoliga GR, Bishop E, Pauszek SJ, Hartwig EJ, Rodriguez LL and Arzt J (2016) Detection of foot-and-mouth disease virus RNA and capsid protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state. *Transboundary and Emerging Diseases* **63**, 152–164.

Stone SS and DeLay PD (1960) Serum and colostral antibody levels in cattle convalescent from foot-and-mouth disease: tests in calves and fetal tissue. *The Journal of Immunology* **84**, 458–462.

Straver PJ, Bool PH, Claessens AMJM and Van Bekkum JG (1970) Some properties of carrier strains of foot-and-mouth disease virus. *Archiv für die Gesamte Virusforschung* **29**, 113–126.

Subramanian BM, Madhanmohan M, Sriraman R, Reddy RC, Yuvaraj S, Manikumar K, Rajalakshmi S, Nagendrakumar SB, Rana SK and Srinivasan VA (2012) Development of foot-and-mouth disease virus (FMDV) serotype O virus-like-particles (VLPs) vaccine and evaluation of its potency. *Antiviral Research* **96**, 288–295.

Sumption K, Rweyemamu M and Wint W (2008) Incidence and distribution of foot-and-mouth disease in Asia, Africa and South America; combining expert opinion, official disease information and livestock populations to assist risk assessment. *Transboundary and Emerging Diseases* **55**, 5–13.

Sutmoller P and Gaggero A (1965) Foot-and mouth diseases carriers. *Veterinary Record* **77**, 968–969.

Sutmoller P and McVicar JW (1976) Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. *Epidemiology and Infection* **77**, 235–243.

Sutmoller P, Barteling SS, Olascoaga RC and Sumption KJ (2003) Control and eradication of foot-and-mouth disease. *Virus Research* **91**, 101–144.

Swaney LM (1976) Susceptibility of a new fetal pig kidney cell line (MVPK-1) to foot-and-mouth disease virus. *American Journal of Veterinary Research* **37**, 1319–1322.

Thomson GR, Vosloo W and Bastos ADS (2003) Foot and mouth disease in wildlife. *Virus Research* **91**, 145–161.

Thurmond MC and Perez AM (2006) Modeled detection time for surveillance for foot-and-mouth disease virus in bulk tank milk. *American Journal of Veterinary Research* **67**, 2017–2024.

Traub E and Mohlmann H (1943) Typenbestimmung bei Maul-und Klauenseuche mit Hilfe der Komplementbindungsprobe I Mitt: Versuche mit Seren und Antigenen von Meerschweinchen. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene: I. Abt. Originale* **150**, 289–299.

Tseng CC and Li CS (2005) Collection efficiencies of aerosol samplers for virus-containing aerosols. *Journal of Aerosol Science* **36**, 593–607.

Uttenthal Å, Parida S, Rasmussen TB, Paton DJ, Haas B and Dundon WG (2010) Strategies for differentiating infection in vaccinated animals (DIVA) for foot-and-mouth disease classical swine fever and avian influenza. *Expert Review of Vaccines* **9**, 73–87.

Vallée H, Carré H and Rinjard P (1926) Vaccination against FMD by means of formalinised virus. *Journal of Comparative Pathology* **39**, 326–329.

Van Bekkum JG, Frenkel HS, Frederiks HHJ and Frenkel S (1959) Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift voor Diergeneeskunde* **84**, 1159–1164.

Verreault D, Moineau S and Duchaine C (2008) Methods for sampling of airborne viruses. *Microbiology and Molecular Biology Reviews* **72**, 413–444.

Vosloo W, Morris J, Davis A, Giles M, Wang J, Nguyen HTT, Kim PV, Quach NV, Le PTT, Nguyen PHN and Dang H (2015) Collection of oral fluids using cotton ropes as a sampling method to detect foot-and-mouth disease virus infection in pigs. *Transboundary and Emerging Diseases* **62**, e71–e75.

Waldmann O and Trautwein K (1926) Experimentelle Untersuchungen über die Pluralität des Maul-und Klauenseuchevirus. *Berl Tierärztl Wochenschrift* **42**, 569–571.

Yilma T (1980) Morphogenesis of vesiculation in foot-and-mouth disease. *American Journal of Veterinary Research* **41**, 1537–1542.

Zhang Z and Bashiruddin JB (2009) Quantitative analysis of foot-and-mouth disease virus RNA duration in tissues of experimentally infected pigs. *The Veterinary Journal* **180**, 130–132.

Zhang ZD and Kitching RP (2001) The localization of persistent foot and mouth disease virus in the epithelial cells of the soft palate and pharynx. *Journal of Comparative Pathology* **124**, 89–94.

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