

## REVIEW

# The Pathogenesis and Diagnosis of Foot-and-Mouth Disease

**S. Alexandersen, Z. Zhang, A. I. Donaldson and A. J. M. Garland**

*Pirbright Laboratory, Institute for Animal Health, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK Working.*

### Contents

Summary . . . . .	2
Introduction . . . . .	2
Pathogenesis . . . . .	3
Minimal doses and routes of infection . . . . .	3
Direct and indirect contact . . . . .	3
Airborne transmission . . . . .	4
Transmission by the oral route . . . . .	5
Nasal instillation and pulmonary inoculation . . . . .	5
Parenteral inoculation (intradermal, intravenous and intramuscular) . . . . .	5
Scarification . . . . .	6
Incubation periods . . . . .	6
Sites of primary infection . . . . .	7
Secondary sites of replication . . . . .	7
Kinetics of viral replication, load and clearance . . . . .	8
Excretion of virus by infected animals . . . . .	9
The antibody response . . . . .	13
Pathogenesis of acute disease . . . . .	13
Clinical signs and development of lesions . . . . .	13
Mechanisms responsible for fever and general depression/dullness, including immune complexes, cytokines and acute phase proteins . . . . .	17
Persistent infection with FMDV: the importance of the carrier problem and mechanisms involved in establishment of persistent infection . . . . .	19
Definition of the pharynx as the site of persistence . . . . .	19
Evidence of persistence at other sites than the pharynx . . . . .	20
Evidence of transmission from carrier animals . . . . .	20
Carriers under field conditions . . . . .	21
Possible mechanisms behind persistence in the pharynx . . . . .	22
Receptors . . . . .	23
Survival of FMDV . . . . .	23
Spread of infection and disease control . . . . .	24
Diagnosis . . . . .	24
Methods for diagnosis . . . . .	24
Tests for antigen/virus/genome . . . . .	25
Antibody detection . . . . .	26
Distinguishing infected from vaccinated stock . . . . .	26
Tests to detect antibodies against the conserved, non-structural proteins of FMDV . . . . .	27
Antibody and antibody isotype concentrations . . . . .	27
Conclusions . . . . .	27
References . . . . .	27

### Summary

The pathogenesis of foot-and-mouth disease (FMD) is reviewed, taking account of knowledge gained from field and experimental studies and embracing investigations at the level of the virus, the cell, the organ, the whole animal and the herd or flock. The review also addresses the immune response and the carrier state in FMD. Progress made in understanding the pathogenesis of the disease is highlighted in relation to developments in diagnosis and methods of control.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Cattle; FMD diagnosis; FMD pathogenesis; FMD virus excretion; FMD virus persistence; foot-and-mouth disease; pig; review article; sheep; viral infection.

### Introduction

Foot-and-mouth disease (FMD) is a severe, clinically acute, vesicular disease of cloven-hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species (Coetzer *et al.*, 1994). The aetiological agent, foot-and-mouth disease virus (FMDV), is classified within the *Aphthovirus* genus as a member of the *Picornaviridae* family (Belsham, 1993), being a non-enveloped, icosahedral virus, 26 nm in diameter, containing positive sense RNA of around 8.4 kb. During intracellular, cytoplasmic replication, viral RNA is translated into a polyprotein that is proteolytically cleaved into 12 structural and non-structural proteins. The complete viral capsid consists of 60 copies of each of the four structural proteins VP1-4, with many critical determinants for infection and immunity inherent in the molecular constituents of the VP1 protein. FMD is classified by l'Office International des Épizooties (OIE; World Organisation for Animal Health) as an OIE List A disease, which, by definition, means that it has the potential for rapid and extensive spread within and between countries and can cause severe economic impact.

The clinical diagnosis of FMD is sometimes difficult, for example in sheep and goats, in which clinical signs are often mild (Callens *et al.*, 1998; Barnett and Cox, 1999; Donaldson and Sellers, 2000; Alexandersen *et al.*, 2002c; Hughes *et al.*, 2002). Moreover, certain strains of the virus may be of low virulence for some species (Donaldson, 1998). In addition, several other viral vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesivirus infection, cannot be distinguished from FMD solely on the basis of clinical findings. Thus, a definitive diagnosis requires laboratory investigation. Given the potential of FMD for rapid spread, it is essential that suspected cases are quickly reported and investigated by means of rapid and accurate tests, so that control

measures can be speedily implemented. Seven distinct serotypes of FMDV, with indistinguishable clinical effects, have been defined, namely types O, A, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. Recovery from infection, or protective vaccination, with one serotype will not protect against subsequent infection with another. Moreover, within a serotype a wide range of strains may occur, some of which may be sufficiently divergent to reduce the efficacy of existing vaccines (Kitching *et al.*, 1989; Kitching, 1998). FMDV has a wide host range, an ability to infect in small doses, a rapid rate of replication, a high level of viral excretion and multiple modes of transmission, including spread by the wind. These features make FMD a difficult and expensive disease to control and eradicate, and one that is much feared by farmers, veterinarians and those associated with livestock production. Countries free of the disease take great precautions to ensure that the virus does not gain entry. Consequently FMD is a major constraint to international trade in livestock and animal products.

The situation is further complicated by the fact that after the acute stage of infection FMDV may cause a prolonged, symptomless, persistent infection in ruminants (so-called "carrier" animals) (Van Bekkum *et al.*, 1959a,b; Suttmoller and Gaggero, 1965; Alexandersen *et al.*, 2002b). The carrier state can occur in convalescent animals or in vaccinated animals exposed to live virus. Recognition of the carrier state and the risk of viral transmission by carrier animals has had a major impact on the design of control and eradication strategies for FMD. The "stamping-out" policy, whereby all susceptible species, both affected and apparently normal, on infected premises are slaughtered in the event of an outbreak, is based mainly on the premise that total slaughter is necessary to eliminate the virus. Were this not to be done the

perception is that some of the animals allowed to survive might be carriers and pose a continuing risk of disease recrudescence. The perceived risk posed by carriers has also had a marked influence on the safeguards taken to manage the risks associated with international livestock movements. The measures, which are mainly directed at animals originating from countries where the disease is either endemic or sporadic, range from a complete embargo to quarantine and testing.

FMD is endemic in large areas of Africa, Asia and South America and has shown an extraordinary ability to cross international boundaries and cause epidemics in previously free areas, as illustrated by the 2001 epidemic in the UK and continental Europe, as well as the outbreaks in the year 2000 in Japan and South Korea (Knowles *et al.*, 2001b). It has been estimated that the direct cost of the UK epidemic in 2001 was £2.75 billion. The indirect costs from the combined losses of agricultural exports and tourist trade are difficult to estimate accurately but probably amounted to an additional £5.25 billion.

In this review the authors examine current knowledge of the pathogenesis of FMD, particularly during acute disease and in the carrier state, and summarize the relevance of recent advances to the diagnosis, surveillance and control of FMD. Particular account has been taken of the following publications: Terpstra, 1972; Garland, 1974; Burrows *et al.*, 1981; Donaldson, 1987; Brown *et al.*, 1992, 1995, 1996; Salt, 1998; Donaldson and Sellers, 2000; Alexandersen *et al.*, 2001, 2002b,c; Hughes *et al.*, 2002.

### Pathogenesis

Susceptible livestock may be infected by FMDV as a result of direct or indirect contact with infected animals or with an infected environment. When infected and susceptible animals are in close proximity, the aerial transfer of droplets and droplet nuclei is probably the most common mode of transmission. Long-range airborne transmission of virus is an uncommon but important route of infection, requiring the chance combination of particular factors, including (1) the animal species, (2) the number and location of the transmitting and recipient animals, and (3) favourable topographical and meteorological conditions. Pathogenesis studies have been carried out in animals infected by simulated natural methods (direct or indirect contact with infected donors or virus aerosols from such donors) or in animals infected by artificial methods, including subcutaneous, intradermal,

intramuscular and intravenous inoculation, intranasal instillation, and exposure to artificially created aerosols. Studies carried out to establish minimum infective doses for the main livestock species, with various serotypes and strains of FMDV delivered by different routes, are summarized in the following section. An important reservation concerning the statistical significance of the numerical values arises as a result of the practical and cost constraints on the number of animals that could be used for the experiments and the number of variables that could be investigated. In addition, the several methods used for titration of virus were of varying sensitivity and may not be directly comparable. The results should therefore be taken as indicators and not as absolute values.

#### *Minimal Doses and Routes of Infection*

*Direct and indirect contact.* The most common mechanism of spread of FMD is by direct contact, which may take the form of (1) mechanical transfer of virus from infected to susceptible animals, the virus entering through cuts or abrasions or through the mucosae, or (2) infection by the deposition of droplets or droplet-nuclei (aerosols) in the respiratory tract of recipient animals. The intact epidermis provides a measure of protection, especially where it is keratinized, but pre-existing traumatic damage or infection may predispose to direct infection. In certain situations, especially with pigs—which are relatively resistant to aerosol exposure (Alexandersen and Donaldson, 2002; Alexandersen *et al.*, 2002a)—contact transmission may be facilitated by physical contact with infected excretions or secretions, including vesicular fluid or vesicular epithelium containing relatively large amounts of FMDV. Moreover, if livestock have pre-existing damage to the integument they are highly susceptible to infection through the damaged skin. Such damage is common in pigs kept on concrete floors, or under intensive conditions which may lead to fighting; in cattle feeding on thorny materials (which penetrate the tissues of the mouth); in dairy cows with teats damaged by milking machines; and in sheep suffering from foot-rot. Contact with virus from infected animals may also occur indirectly, via contaminated personnel, vehicles, and all classes of fomites. Activities such as shearing, de-worming and rounding up for lambing or for clinical examination and blood sampling (common procedures during FMD epidemics), increase the risk of indirect spread of the virus and of its entry through traumatized epidermis.

*Airborne transmission.* Under certain climatic and meteorological conditions, the short-distance droplet and droplet nuclei transmission referred to above may be extended to long-range airborne transmission. This is especially significant when pigs are the source of infection because, of the major domesticated species, the pig liberates the largest quantities of airborne virus (Sellers and Parker, 1969; Donaldson *et al.*, 1970, 1982a; Donaldson and Ferris, 1980; Alexandersen and Donaldson, 2002; Alexandersen *et al.*, 2002a). Ruminants excrete less virus in their breath but, in contrast to pigs, are highly susceptible to infection by the respiratory route. Thus, ruminants may be infected experimentally by airborne exposure to as little as 10 tissue culture 50% infective doses (TCID<sub>50</sub>) whereas pigs require more than 10<sup>3</sup> TCID<sub>50</sub>. Furthermore, to infect pigs consistently the dose has to be delivered at a high concentration (Donaldson *et al.*, 1970; Donaldson and Ferris, 1980; Donaldson, 1986; Gibson and Donaldson, 1986; Donaldson *et al.*, 1987; Donaldson and Alexandersen, 2001; Alexandersen and Donaldson, 2002; Alexandersen *et al.*, 2002a). Consequently, the most likely pattern of airborne FMD spread is from pigs to cattle and sheep downwind. Cattle are more readily infected than sheep by the airborne route because, being larger, they inhale (i.e., “sample”) more air in a given time. FMDV isolates vary greatly in the distance over which airborne spread can occur. Some isolates, for example the type O UK 2001 strain, are unlikely to be spread more than about 20 km by the wind, even when many infected pigs provide the source (Donaldson *et al.*, 2001; Alexandersen and Donaldson, 2002; Donaldson and Alexandersen, 2002). However, other isolates, in particular C Noville, have the potential to spread up to about 300 km by the wind (Gloster *et al.*, 1981, 1982; Donaldson *et al.*, 1982a,b; Sorensen *et al.*, 2000, 2001). Such long-distance airborne spread is likely to occur only in circumstances where the biological and physical loss of infectivity in the plume is minimal due to the specific climatic and topographical conditions. Favourable conditions include a relative humidity of 55% or more, and minimal mixing of the air by turbulence and convection. Such conditions are typically seen when there is a continuous steady or slight wind, cloud cover and level topography. For further details see Donaldson *et al.* (2001), Alexandersen and Donaldson (2002) and Donaldson and Alexandersen (2002).

Natural aerosols from infected animals contain large, medium and small particles excreted as

droplets and droplet nuclei in the breath. The respiratory tract is the source of the infectious particles, but the mechanism of virus release is not understood. From pigs, 35–70% of the infectivity is associated with large particles (>6 µm diameter), 20–40% with medium sized particles (3–6 µm diameter) and 10–25% with small particles (<3 µm diameter). When inhaled by recipient animals a proportion of these particles will be deposited in the respiratory system, the sites of deposition being determined mainly by the diameter and mass of the particles. Large particles will be deposited in the upper respiratory tract (nares), medium-sized particles in the middle to upper respiratory tract (pharynx, trachea, bronchi) and small particles in the lower regions (small bronchioles and alveoli) (Hatch and Gross, 1964; Sellers and Parker, 1969; Donaldson *et al.*, 1970, 1987; Donaldson and Ferris, 1980; Alexandersen, unpublished). The regions in the respiratory tract of recipient animals that will be exposed to virus initially will depend on the distance between the recipient animals and the source of airborne virus, and on the amount of air turbulence. Larger droplets will be affected by gravity and will tend to sediment rapidly. In still air the rate of fall-out of such droplets will be high, but turbulence will keep them suspended for longer. Particles of less than 6 µm diameter will not be greatly affected by gravity and therefore can be transported over long distances (Gloster *et al.*, 1981). These are the particles which contain high amounts of FMDV and are most likely to be deposited in the upper and middle to upper regions of the respiratory tract. Particles landing in the nares will be taken backwards towards the pharynx along the mucociliary escalator. Similarly, smaller particles landing in the trachea and bronchi will be taken upwards towards the pharynx. The deposition and concentration of virus in the pharynx (a predilection site for FMDV replication; see later) is therefore determined by the particle size. After initial replication in the pharynx, the virus spreads through the lymphoid system into the general circulation. In contrast, the pathogenesis of infection in animals exposed to artificially generated aerosols of small (<3 µm) particle size will be different, since the initial route of infection will be mainly through the walls of the lower respiratory system and thence to the pulmonary circulation and general circulation, leading to infection and replication at distant predilection sites (Donaldson *et al.*, 1987).

In addition to excretion of virus in the breath of infected animals, aerosols may be created by

the splashing of infected milk and urine, by the use of high pressure hoses to clean areas contaminated with infectious secretions, excretions and lesion materials, and by the process of spraying infected slurry on pastures. However, the infectivity of, and therefore risk of spread by, such aerosols is likely to be much less than that of aerosols exhaled by infected animals.

*Transmission by the oral route.* The origin of FMD epidemics in countries normally free from the disease is frequently difficult to identify with certainty, but several recent outbreaks have been linked to the entry of virus in contaminated material which has subsequently been fed to animals. For example, the South Africa 2000 and UK 2001 epidemics have been attributed to the feeding of unheated waste food to pigs, and the Japan 2000 epidemic to the feeding of contaminated fodder (Knowles *et al.*, 2001b). It should be noted that animals are relatively insensitive to experimental infection by the oral route the dose for pigs being about  $10^4$ – $10^5$  and for ruminants about  $10^5$ – $10^6$  TCID<sub>50</sub> (Sellers, 1971). These doses are much higher than those required to infect by the airborne route (Donaldson, 1987). It should also be noted, however, that animals with abrasions of the epithelium in and around the mouth may be infected by smaller doses (Donaldson, 1987). Sharp objects, such as pieces of bone, may therefore facilitate infection by contaminated waste food.

*Nasal instillation and pulmonary inoculation.* These methods have been used in experimental studies to initiate infection. Generally the dose that will infect by nasal instillation is much larger than that given as an aerosol, i.e., around  $10^4$ – $10^5$  TCID<sub>50</sub> for cattle and sheep (McVicar and Suttmoller, 1976). As described previously for the airborne route of infection, the portal of entry is probably the pharynx (McVicar and Suttmoller, 1976). However, after nasal instillation only a small proportion of the dose is likely to reach the pharynx, since most of it will either be swallowed or will exit via the nares. As far as the authors are aware, the susceptibility of pigs to nasal instillation is unknown, but the high resistance of pigs to airborne infection may suggest that it is relatively low. Conversely, however, as pigs are relatively susceptible to oral infection, it is possible that intranasally instilled virus could infect through the mouth, especially when there are pre-existing oral abrasions.

Various authors have hypothesized that the lung is a specific site for entry and replication of the virus (Eskildsen, 1969; Suttmoller and McVicar, 1976; Brown *et al.*, 1996). There is no doubt that infection

can be initiated by an artificial aerosol deposited in the lung or following the introduction of virus directly into a bronchus or bronchiole. However, in our opinion it is possible that virus administered by these methods can reach the circulation directly without local replication, and thereafter initiate infection at the usual target sites (see later). We consider that the histopathological pulmonary lesions reported in the literature were unrelated to FMD, and more likely represented a reaction to the experimental inoculum or were caused by an unrelated, intercurrent respiratory infection. In our experiments, we have consistently failed to find any indication of significant replication of FMDV in the lung. Pigs infected by the airborne route (by a natural aerosol from infected pigs or by direct contact with infected pigs) showed no signs of pulmonary replication (Alexandersen *et al.*, 2001). Similarly, in a study in cattle infected by needle-inoculation no indication was found of viral replication in the lung (see below). Other workers have found no evidence to support the proposal that the lung is a predilection site for initial infection and replication (Burrows *et al.*, 1981). During the acute phase of the disease virus can be found in virtually all organs as a consequence of the high level of viraemia, and this includes the lungs. Consequently, organs should be regarded as sites of active viral replication only when they show a higher concentration of virus or viral RNA than that found in blood sampled at the same time.

*Parenteral inoculation (intradermal, intravenous and intramuscular).* These routes of infection have been used in experimental studies and have occasionally been incriminated as causing iatrogenic disease due to the use of contaminated instruments (e.g., needles) or medicinal products. Examples include the injection of an FMDV-contaminated pituitary extract and administration of FMD vaccines containing live virus as a consequence of the use of inappropriate inactivants (Beck and Strohmaier, 1987).

In experimental studies on FMD, for example when donors are required for pathogenesis studies or when vaccine potency is to be measured, the animals are generally infected by intradermal/subdermal injection of virus, as this is dependable for establishing infection. This route of infection targets the highly susceptible epithelial regions of the tongue in cattle or of the coronary bands or heel bulbs of the feet in sheep and pigs, respectively (Henderson, 1949, 1952; Burrows, 1966b, 1968b). These are sensitive and reliable routes for establishing infection, usually with a very short incubation period. Although artificial, these methods simulate



infection through damaged skin as described below, by-passing the protective effect of the intact integument. The dose to establish infection may be as low as 100 TCID<sub>50</sub>, although under experimental conditions a dose of around  $10^4$ – $10^5$  TCID<sub>50</sub> may be required to give consistent results. Doses from 5 to 10-fold lower have been cited in the literature, but without details of the assay systems used (Sellers, 1971).

Intravenous inoculation also results in a relatively short incubation period, but this method appear to be less efficient and more variable than the intradermal/subdermal methods referred to previously (Henderson, 1952). Intramuscular inoculation, which has also been used, is a relatively inefficient method and may require a dose of  $10^4$  TCID<sub>50</sub> or more (Burrows *et al.*, 1981; Donaldson *et al.*, 1984). Interestingly, intramuscular inoculation does not appear to create the carrier state in immunized ruminants (Sutmoller *et al.*, 1968), probably because the virus is prevented from reaching the pharyngeal area by antibodies present at the site of inoculation and in the circulation.

**Scarification.** Another efficient method of infection is through damaged skin. Although now rarely used experimentally, this is carried out by applying a suspension of virus to scarified skin. The protective effect of intact integument was shown in an experiment in which exposure of the bovine tongue to  $10^{7.8}$  infectious units for 10 min did not lead to FMD unless the tongue was first scratched with a needle (Cottral *et al.*, 1965). This method resembled infection through damaged skin, which may—as already discussed—be a significant route of natural transmission in animals with pre-existing lesions due to trauma or intercurrent disease.

The estimated minimum doses to infect cattle, sheep or pigs by some of the routes described above are summarized in Table 1. It is emphasized that these are not absolute values but represent estimates based on experiments in which small groups of

animals and different methods were used; the results are therefore not directly comparable.

### Incubation Periods

The incubation period of an infectious disease is defined as the time interval between exposure to an infective dose and first appearance of clinical signs. The incubation period for FMD is highly variable, and depends on the strain and dose of virus, the route of transmission, the animal species and the husbandry conditions. The incubation period for farm-to-farm airborne spread ranges from 4 to 14 days (Sellers and Forman, 1973) and this is also the normal range for farm-to-farm spread by indirect contact. The incubation period for farm-to-farm spread resulting from direct contact may range from 2 to 14 days (Garland and Donaldson, 1990). For within-farm spread the period is generally 2–14 days but may be as short as 24 h, especially in pigs and under very high challenge conditions. When spread is occurring within a herd or flock, the typical incubation period is 2–6 days, although, as mentioned above, under certain conditions it may be as short as 1 day or as long as 14 days. These ranges in incubation period are supported by both field and experimental observations (Alexandersen *et al.*, 2003a,b). Under experimental conditions the mean incubation period was 3.5 days for continuous, direct cattle-to-cattle contact and 2 days for intensive sheep-to-sheep contact (Alexandersen *et al.*, 2002c, 2003b.; Garland, unpublished). Pigs were readily infected by direct pig-to-pig contact exposure, with a mean incubation period of 1–3 days depending on the intensity of contact (Alexandersen *et al.*, 2003b.). These differences confirm the strong relation between dose and length of incubation, i.e., the higher the dose or the intensity of contact the shorter is the incubation period. Under field conditions the dose of FMDV will be

**Table 1**  
Selected estimated minimum doses\* for various species and routes of exposure

Species	Inhalation	Intradermal	Intramuscular	Nasal instillation	Oral
Cattle	10	100	$10^4$	$10^4$ – $10^5$	$10^5$ – $10^6$
Sheep	10	100	$10^4$	$10^4$ – $10^5$	$10^5$ – $10^6$
Pigs	> 800	100	$10^4$	Unknown	$10^4$ – $10^5$

\*The estimated minimum doses are those reported to cause *clinical* disease. It is emphasized that these are not absolute values but represent estimates based on different experiments that are not necessarily directly comparable. It is possible that even smaller doses might produce infection if large numbers of animals were exposed. Doses are given as TCID<sub>50</sub> (bovine thyroid tissue culture 50% dose end-point estimates). For further information see the text and associated references. It should be noted that for intradermal and intramuscular inoculation, doses from 5 to 10 fold lower are cited in the literature, but without details of the assay systems used (Sellers, 1971).

influenced by many factors, especially the stocking density (i.e., intensive or extensive management), whether the animals are housed and, if so, whether in well or poorly ventilated surroundings. The extent to which animals are handled, especially around the nostrils and mouth, will also influence the incubation period. Such handling is common when, for example, sheep are de-wormed or when farmers, veterinarians or livestock dealers examine animals on farms or at markets. Other common farming practices such as the gathering of sheep for shearing, dipping, vaccination, transport, marketing etc. will also accelerate the rate of spread of virus in an infected flock or herd.

#### *Sites of Primary Infection*

Several lines of evidence point to the pharyngeal area as the usual primary site of infection except, as already mentioned, for those occasions when the virus gains direct entry into the skin or mucosa through cuts or abrasions. The importance of the pharynx has been demonstrated for cattle by means of both direct and indirect contact infection, intranasal instillation and airborne exposure (Garland, 1974; McVicar and Suttmoller, 1976; Burrows *et al.*, 1981). The dorsal surface of the soft palate and the roof of the pharynx, just above the soft palate, are sites of particular significance. With “real-time” reverse transcription-polymerase chain reaction (RT-PCR) and in-situ hybridization we have demonstrated these same primary sites of infection for pigs infected by contact or by airborne virus, and also for cattle infected by contact or by needle-inoculation (Zhang and Kitching, 2000, 2001; Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001; Zhang and Alexandersen, unpublished). In sheep, Burrows (1968b) reported that the tonsils played a role of particular importance in the initial infection. This may be explained, however, by the fact that in sheep the tonsils are located immediately above the dorsal soft palate, resulting in close physical contact between the two surfaces; in addition, the epithelium covering this part of the tonsil may be of a transitional type similar to that on the dorsal surface of the soft palate.

The epithelial cells on the dorsal soft palate, the roof of the pharynx and part of the tonsil are thought to play a special role in primary infection. Many of the epithelia of the oral cavity are of the stratified squamous type and are, moreover, cornified (i.e., have a layer of dead cells overlying the outer surface). In contrast, the anatomical regions mentioned above are covered by special, stratified squamous epithelia which are non-cornified and

thus have live cells throughout their substance, from the basal layer to the surface (see later). Interestingly, the special epithelial cells in this region of the pharynx appear also to be responsible for supporting persistent infection in carrier animals. In contrast, virus appears to be completely cleared from other sites commonly infected in the acute stage, including the predilection sites of generalized vesiculation, which are located principally in the cornified, stratified squamous epithelium of the skin, feet and mouth.

Virus may be demonstrated in the pharynx for 1–3 days before a viraemia can be detected (Burrows, 1968a; McVicar and Suttmoller, 1976; Burrows *et al.*, 1981; Alexandersen *et al.*, 2002b,c; Zhang and Alexandersen, unpublished; Garland, unpublished). Viraemia usually lasts for 4–5 days (Cottrell and Bachrach, 1968; Alexandersen *et al.*, 2002c, 2003b; Garland, unpublished) and is the means by which virus is distributed to secondary sites (see later).

Under certain circumstances, for example after injection of virus into the skin or tongue or when infection takes place through damaged skin, replication will take place at the site of entry. After this initial replication and spread through regional lymph nodes (Henderson, 1948) and into the circulation, a number of secondary sites will be infected, especially the skin (both with and without hair) and the epithelia of the tongue and mouth, i.e., cornified epithelia, where the main viral amplification occurs. The kinetics of infection are discussed below. As mentioned earlier, we have found no evidence to suggest that the lung is either a primary or secondary site of viral amplification. Nasal mucosa has also been suggested as a site of initial replication (Korn, 1957), but although nasal fluid contains significant amounts of virus during the prodromal and acute clinical phases (see later), we have found no evidence to support this hypothesis.

#### *Secondary Sites of Replication*

The earliest sites of FMDV infection and replication in contact-exposed animals appear to be in the pharynx, as detailed above. Viral replication may reach a peak as early as 2–3 days after exposure (Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001). Recent data indicate that after initial replication the virus enters through regional lymph nodes and into the bloodstream. The greater part of the viral amplification occurs subsequently within the cornified stratified squamous epithelia of the skin (including the feet and mammary gland) and

mouth (including the tongue), or in the myocardium of young animals. Although some viral replication occurs in the epithelia of the pharynx, it is much less than in the skin and mouth during the acute phase of the disease. Moreover, by comparison with the skin and mouth, other organs (e.g., salivary glands, kidneys, liver, spleen, lymph nodes, lung and nasal mucosa) produce no more than negligible amounts of virus (Hess *et al.*, 1967; Burrows *et al.*, 1981; Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001; Zhang and Alexandersen, unpublished). It has been suggested that a secondary phase of replication occurs in lymph nodes. Some evidence from in-vitro studies has indicated that FMDV infectivity may persist in macrophages for 10–24 h (Rigden *et al.*, 2002). However, experimental investigations *in vivo* (Cottral *et al.*, 1963; Burrows *et al.*, 1981) and more recent studies suggest that lymph nodes as well as lymphocytes and macrophages (including alveolar macrophages) play little or no part in FMDV replication (Alexandersen *et al.*, unpublished) and that any virus present in lymphoid organs is produced elsewhere, i.e., in the pharynx and the epithelia of the mouth and skin.

#### *Kinetics of Viral Replication, Load and Clearance*

To elucidate the pathogenesis of FMD and spread of the causative virus it is important to understand the quantitative aspects of the acute phase of the infection. Such aspects have been studied in great detail in cattle and to a limited extent in sheep, generating data on the concentration of virus in various organs in relation to the time after infection (Hess *et al.*, 1967; Cottral and Bachrach, 1968; Burrows, 1968a; McVicar *et al.*, 1970; McVicar and Suttmoller, 1971; Sellers, 1971; Garland, 1974; Burrows *et al.*, 1981; Alexandersen *et al.*, 2002c).

With the exception of detailed studies on aerosol excretion in pigs (Donaldson *et al.*, 1970; Donaldson, 1979, 1986; Donaldson and Ferris, 1980) the quantitative aspects of porcine infection have been somewhat limited (Terpstra, 1972). However, we recently described the quantitative aspects (viral load) in various tissues of contact-infected pigs at 1–4 days after infection (Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001). We have also obtained preliminary results of studies of the viral loads in infected cattle (Zhang and Alexandersen, in preparation). These investigations confirm that early replication takes place in the pharynx and that the main amplification of virus subsequently takes place in the epithelia of the mouth and skin. It is to be noted that while vesicular epithelia

contained the highest concentration of virus, apparently normal skin, both hairy and hairless, also contained significant amounts (Alexandersen *et al.*, 2001).

On the basis of current knowledge we have proposed the following model for the progression of infection (Alexandersen *et al.*, 2002b). Primary exposure to live virus results in the establishment of infection and accumulation of FMDV in the non-cornified epithelium of the pharyngeal area. Virus then spreads through regional lymph nodes and via the bloodstream to cornified epithelial cells of the skin and mouth, resulting in several cycles of viral amplification and spread.

The host reaction, including antibody production (see later), can be detected from 3 to 4 days after the first clinical signs and usually clears the virus, except in those ruminant animals that develop a persistent infection of the pharyngeal region (see below). Immunity to FMDV is primarily mediated by circulating antibodies that are relatively efficient in clearing virus from the circulation. However, clearance of virus (as assessed by the examination of swabs) from “peripheral/external” sites, such as the nasal and oral surfaces is less efficient. Still less efficient is clearance (as assessed by the examination of probang samples) from the oesophageal–pharyngeal region. Similarly, virus may remain in vesicular epithelium for up to 10–14 days, detectable for longer periods in foot lesions than in mouth lesions (Oliver *et al.*, 1988). Recovery from infection and the protection induced by inactivated vaccines or passively transferred antibodies against the development of clinical FMD are closely correlated with the concentration of circulating antibodies; such antibodies, however, do not protect against local pharyngeal infection and are ineffective in clearing virus from the pharynx of carrier ruminants (Brown and Cartwright, 1960; Hess *et al.*, 1967; McVicar and Suttmoller, 1974, 1976; Francis and Black, 1983; Francis *et al.*, 1983; Black *et al.*, 1984; Hamblin *et al.*, 1987; McCullough *et al.*, 1992; Aggarwal *et al.*, 2002; Alexandersen *et al.*, 2002b). In infected pigs the virus is totally cleared (undetectable even at the RNA level) in 3–4 weeks or less while, by contrast, around 50% or more of cattle will become carriers (Salt, 1993, 1998; Donn *et al.*, 1994; Prato Murphy *et al.*, 1994; Woodbury *et al.*, 1995; Murphy *et al.*, 1999; Zhang and Kitching, 2000, 2001; Alexandersen *et al.*, 2002b).

Most knowledge about the quantitative aspects of infection has been gained from studies of viral excretion and viral load in blood or serum and is described in more detail below.



*Excretion of Virus by Infected Animals*

All secretions and excretions become infectious during the course of the disease and some contain significant titres of virus before the development of clinical signs (see [Tables 2,3 and 4](#)). Thus saliva, nasal and lachrymal fluid, milk and expired breath may contain virus during the prodromal period. Urine and faeces also contain virus but to a lesser extent. It appears that faeces *per se* contain only small amounts of virus ([Parker, 1971](#)), but once voided they are likely to be contaminated further by admixture with desquamated lesion material, vesicular lymph, saliva, milk and urine. Since preputial lesions are sometimes present it is possible that these are the origin of infectivity in urine. In experimental studies at Pirbright ([Garland, 1974](#)) groups of steers were indirectly exposed to infected pigs and sampled daily for virus isolation and assay in primary bovine (calf) thyroid cells (BTY), this being the most sensitive tissue culture system available for the detection of infectivity ([Snowdon, 1966](#)). It was found that oral saliva and probang samples from the pharynx were the first to contain

new virus ([Tables 2 and 3](#)). This finding was subsequently confirmed by the use of “real-time” RT-PCR to examine samples from cattle exposed to infected donor cattle; nasal and mouth swabs, however, were also positive at an early stage ([Alexandersen \*et al.\*, 2003b](#)).

Studies in sheep demonstrated that virus was detectable in the breath 1-2 days before the appearance of clinical signs ([Sellers and Parker, 1969](#)) and subsequently in the blood, followed by the nasal fluid, as indicated in [Fig. 1](#) ([Alexandersen \*et al.\*, 2002c](#)). By contrast, the peak of airborne viral excretion in cattle and pigs occurred during the viraemic phase, after early generalized lesions had developed. The average peak amounts of airborne virus recovered per 24 h from animals experimentally infected with FMDV type O, strain UK 2001, were  $10^{4.3}$  TCID<sub>50</sub> for a sheep or a heifer and  $10^{6.1}$  TCID<sub>50</sub> for a pig ([Donaldson \*et al.\*, 2001](#); [Alexandersen and Donaldson, 2002](#); [Alexandersen \*et al.\*, 2002c](#)). These findings confirm the pattern of airborne viral excretion found with several other strains of FMDV. However, the differences found

**Table 2**  
**Concentrations of FMDV type O (strain BFS 1860) in the secretions and excretions of three steers sampled for 10 days after indirect exposure to diseased pigs (from [Garland, 1974](#))**

Steer no.	Sample	Results (bovine thyroid cells ID <sub>50</sub> /ml or g) on stated days after exposure										
		0	1	2	3	4	5	6	7	8	9	10
HR8	NS	0.0	0.0	1.5	4.0	5.5	5.0	4.5	2.5	0.0	Tr	0.0
	OS	0.0	Tr	2.0	4.5	5.5	6.0	5.5	3.0	1.0	0.0	0.0
	PhF	0.0	0.0	4.5	4.0	6.0	4.5	4.5	5.0	1.5	1.0	1.5
	F	0.0	0.0	0.0	0.0	Tr	0.0	1.5	0.0	0.0	0.0	0.0
	U	0.0	–	0.0	0.0	1.0	1.5	1.5	0.0	–	0.0	0.0
	PS	0.0	0.0	Tr	1.5	3.5	4.0	4.0	2.5	0.0	0.0	0.0
	Ser	0.0	0.0	0.0	1.2	5.2	5.2	2.2	0.0	0.0	0.0	0.0
HR9	NS	0.0	Tr	0.0	1.5	5.0	5.0	4.5	3.0	1.0	0.0	0.0
	OS	0.0	1.5	2.0	3.5	5.5	6.0	5.5	1.5	0.0	0.0	0.0
	PhF	0.0	2.0	5.5	4.0	4.5	4.5	3.5	2.5	1.5	1.0	1.0
	F	0.0	0.0	0.0	Tr	1.5	Tr	0.0	1.5	0.0	0.0	0.0
	U	0.0	0.0	–	1.0	2.0	0.0	2.5	0.0	0.0	0.0	0.0
	PS	0.0	0.0	Tr	2.5	4.0	4.5	4.0	1.0	0.0	0.0	0.0
	Ser	0.0	0.0	Tr	1.2	4.2	5.2	1.5	0.0	0.0	0.0	0.0
HR11	NS	0.0	2.0	4.0	5.0	3.5	2.0	0.0	0.0	0.0	0.0	0.0
	OS	0.0	3.5	5.8	5.5	6.0	4.0	2.3	0.0	0.0	0.0	0.0
	PhF	0.0	6.5	7.0	5.0	4.5	3.5	2.5	2.0	0.0	1.0	1.0
	F	0.0	0.0	1.5	0.0	Tr	0.0	0.0	0.0	0.0	0.0	0.0
	U	0.0	–	1.0	1.0	0.0	–	1.0	0.0	0.0	0.0	0.0
	PS	0.0	1.5	3.5	3.5	2.0	0.0	1.0	0.0	0.0	0.0	0.0
	Ser	0.0	3.0	3.0	4.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0

NS, nasal secretion; OS, oral saliva; PhF, pharyngeal fluid; F, faeces; U, urine; PS, preputial swab; Ser, serum. – No sample; Tr, trace of virus (one or two tubes showing cytopathogenic effect at lowest dilution tested); 0.0, no virus detected at lowest dilution tested (see below). Lesions were first detected clinically at 4, 4 and 2 days post-exposure in steers HR8, HR9 and HR11, respectively. The three cattle were infected by holding them for 1 h in a loose-box immediately after it had been vacated by a group of six pigs, infected 2 days earlier by the injection of FMDV, and showing clinical signs. The cattle were then transferred to a clean loose-box. The animals were sampled just before infection and then daily at approximately 24-h intervals after exposure to infection. Samples were assayed in primary bovine thyroid cells (10-fold dilution steps and four cell culture tubes per dilution). The lowest dilutions tested were 1 in 1 for all samples, except faeces (1 in 10) and preputial swabs (*c.* 1 in 5).

**Table 3**  
**Mean concentrations of FMDV in the secretions and excretions of cattle before and after the first appearance of macroscopical lesions resulting from indirect contact with infected pigs**

Sample	Virus type	<i>Results (bovine thyroid cells ID50/ml or g) on stated days before or after first appearance of macroscopical lesions</i>											
		− 4	− 3	− 2	− 1	0	1	2	3	4	5	6	7
Ser	O	–	–	0.2	3.2	4.9	5.3	2.6	–	–	–	–	–
	C	–	–	–	3.9	5.9	6.1	0.4	–	–	–	–	–
PhF	O	–	0.3	2.8	5.6	6.1	5.5	4.1	4.3	2.6	1.9	0.9	1.3
	C	–	2.2	4.7	6.8	7.1	6.3	5.5	5.1	3.0	2.1	2.4	2.5
OS	O	–	0.3	1.6	4.7	6.5	6.4	5.4	3.6	1.8	0.5	–	–
	C	–	1.4	5.3	7.0	7.5	6.7	6.3	4.4	3.0	1.5	0.3	–
NS	O	–	–	0.7	3.3	5.6	5.6	4.5	3.1	1.2	0.1	–	–
	C	–	0.9	3.9	5.5	7.1	6.5	5.7	2.7	1.0	0.4	–	–
LS	O	–	–	0.2	3.3	4.4	5.4	5.3	4.5	3.3	1.8	–	–
	C	–	–	0.6	2.0	3.6	5.0	3.3	0.9	–	–	–	–
U	O	–	–	–	0.5	2.4	2.0	1.9	1.4	1.5	–	–	–
	C	–	–	0.8	1.5	2.3	2.0	1.9	1.4	1.5	–	–	–
F	O	–	–	–	0.5	1.5	0.5	0.6	1.0	0.7	–	–	–
	C	–	–	–	1.1	1.9	0.7	0.2	–	–	–	–	–

The table shows a composite set of averaged results for samples from three separate experiments, each with a group of three steers infected with either type O1 (strain BFS 1860), type O1 (strain Swiss 1/66), or type C (strain Noville). The method of exposure of the animals, the method of infectivity assay for the samples, and the symbols used are as for [Table 2](#), apart from the use of LS to denote lachrymal secretion.

between ruminants and pigs in respect of the peak amounts excreted were considerably less for strain UK 2001 than for some other strains ([Sellers and Parker, 1969](#); [Donaldson \*et al.\*, 1970, 1982a\). When data for the UK 2001 virus were used as input to simulate airborne FMDV plumes downwind of hypothetically infected premises, under optimal conditions for airborne spread, the results confirmed again that the risk of airborne spread is greatest for cattle downwind of premises containing infected pigs \(\[Donaldson \\*et al.\\*, 2001\\). For example, cattle would be at risk if located 2 km downwind from a farm containing 100 infected pigs excreting maximally; if, however, the source farm contained 100 infected cattle or sheep excreting maximally, the predicted distance of airborne spread would be around 200 m or less. These distances should be taken as estimates, not absolutes, since some of the parameters in the model were variable or not well understood, in particular the distribution of infectivity in individual droplets and droplet nuclei, the probability of infection at low doses \\(\\[Manuel-Leon and Casal, 2001\\\) and the possible effects of cumulative doses. Furthermore, the behaviour of airborne particles in plumes is complex and their dispersion is not homogeneous \\\(J. Gloster, personal communication\\\). Consequently, it is possible that infectious doses may travel further than predicted by the model.\\]\\(#\\)\]\(#\)](#)

Virus is also excreted in milk and semen ([Burrows, 1968a\) from shortly before clinical](#)

signs appear and through the clinical phase, in a pattern that largely mirrors the viraemia profile. Large amounts of virus are excreted in vesicular fluid, in desquamated vesicular epithelium and, in cattle, also in saliva ([Hyslop, 1965](#); [Scott \*et al.\*, 1966\). There is also excretion, but to a much lesser extent, in faeces \(\[Burrows, 1968a\]\(#\); \[Parker, 1971\]\(#\); \[Garland, 1974\\), in a pattern that also reflects the peak of viraemia, lesions and clinical disease. A sharp decline in viral excretion and load occurs around day 4–5 of clinical disease, when a significant circulating antibody response is detectable.\]\(#\)](#)

The overall pattern of viral excretion for cattle is illustrated in [Tables 2 and 3](#). In addition, [Table 4](#) cites from the literature the maximum infectivity titres for secretions and excretions from cattle with FMD. It should be noted, however, that because these data were obtained with different methods, viral strains and assay systems, the figures are not directly comparable. Nevertheless, the relative values cited are useful indicators of the high infectivity attained by all secretions and excretions, and the consequent massive environmental contamination. [Table 4](#) also shows a theoretical value for the total daily amount of viral infectivity in any given secretion or excretion; this value is based on the amount of each fluid normally produced per day, and on the assumption that the entire daily output is contaminated to the maximum level cited. Such fluids are of importance in mechanical spread, for example, when people become

**Table 4**  
**Selected recorded maximum and calculated theoretical total infectivity titres of some secretions and excretions during the course of FMD in cattle**

Secretion or excretion	Volume or weight*	Virus strain	Maximum recorded viral titre	Theoretical total viral content <sup>†</sup>	Reference
Blood or serum	30 litres	A119	10 <sup>5.8</sup> /ml	10 <sup>10.3</sup>	<b>A</b>
		O Canefa-9	10 <sup>5.6</sup> /ml	10 <sup>10.1</sup>	<b>B</b>
		Various	10 <sup>6.0</sup> /ml	10 <sup>10.5</sup>	<b>C</b>
		O BFS 1860	10 <sup>5.2</sup> /ml	10 <sup>9.7</sup>	<b>E</b>
		O Swiss 1/66	10 <sup>6.8</sup> /ml	10 <sup>11.3</sup>	<b>E</b>
		A119	10 <sup>6.5</sup> /ml	10 <sup>11.0</sup>	<b>E</b>
		C Noville	10 <sup>7.8</sup> /ml	10 <sup>12.3</sup>	<b>E</b>
Lachrymal secretion	Unknown	O Canefa-2	10 <sup>7.0</sup> /sample	...	<b>F</b>
		O Swiss 1/66	10 <sup>6.3</sup> /sample	...	<b>F</b>
		C Noville	10 <sup>6.1</sup> /sample	...	<b>F</b>
Nasal secretion	Unknown	Various	10 <sup>7.7</sup> /g	...	<b>C</b>
		O BFS 1860	10 <sup>5.5</sup> /ml	...	<b>E</b>
		O Swiss 1/66	10 <sup>7.3</sup> /ml	...	<b>E</b>
		A119	10 <sup>6.0</sup> /ml	...	<b>E</b>
		C Noville	10 <sup>8.3</sup> /ml	...	<b>E</b>
		Various	10 <sup>8.0</sup> /ml	10 <sup>13.3</sup>	<b>C</b>
Oral saliva	98–190 litres per day	O Israel 1/63	10 <sup>8.5</sup> /ml	10 <sup>13.8</sup>	<b>G</b>
		O BFS 1860	10 <sup>6.7</sup> /ml	10 <sup>12.0</sup>	<b>D</b>
		O BFS 1860	10 <sup>6.0</sup> /ml	10 <sup>11.3</sup>	<b>E</b>
		O Swiss 1/66	10 <sup>7.8</sup> /ml	10 <sup>13.1</sup>	<b>E</b>
		A 119	10 <sup>7.0</sup> /ml	10 <sup>12.3</sup>	<b>E</b>
		C Noville	10 <sup>8.8</sup> /ml	10 <sup>14.1</sup>	<b>E</b>
		O BFS 1860	10 <sup>7.4</sup> /ml	10 <sup>12.7</sup>	<b>D</b>
		O BFS 1860	10 <sup>7.0</sup> /ml	10 <sup>12.3</sup>	<b>E</b>
		O Swiss 1/66	10 <sup>7.8</sup> /ml	10 <sup>13.1</sup>	<b>E</b>
		A 119	10 <sup>7.3</sup> /ml	10 <sup>12.6</sup>	<b>E</b>
Pharyngeal fluid (probang samples)	Probably as for saliva, (98–190 litres per day)	C Noville	10 <sup>8.3</sup> /ml	10 <sup>13.6</sup>	<b>E</b>
		O Canefa-2	10 <sup>4.1</sup> /g	10 <sup>8.7</sup>	<b>F</b>
		O BFS 1860	10 <sup>2.0</sup> /g	10 <sup>6.7</sup>	<b>D</b>
		O Swiss 1/66	10 <sup>3.0</sup> /g	10 <sup>7.7</sup>	<b>E</b>
		A 119	10 <sup>2.0</sup> /g	10 <sup>6.7</sup>	<b>E</b>
Faeces	14–45 kg per day	C Noville	10 <sup>3.3</sup> /g	10 <sup>8.0</sup>	<b>E</b>
		A119	10 <sup>4.9</sup> /ml	10 <sup>9.2</sup>	<b>H</b>
		O M11	10 <sup>4.6</sup> /ml	10 <sup>8.9</sup>	<b>H</b>
		O BFS 1860	10 <sup>2.5</sup> /ml	10 <sup>6.8</sup>	<b>E</b>
		O Swiss 1/66	10 <sup>5.5</sup> /ml	10 <sup>9.8</sup>	<b>E</b>
Urine	8.8–22 litres per day	A 119	10 <sup>2.5</sup> /ml	10 <sup>6.8</sup>	<b>E</b>
		C Noville	10 <sup>3.3</sup> /ml	10 <sup>7.6</sup>	<b>E</b>

A: From [Cottral and Bachrach \(1968\)](#). Titre in mouse ID50 /ml or g. Cattle infected by tongue inoculation. Report gives details of types and strains not listed in Table 4. B: From [Cottral \(1969\)](#). Titre in mouse ID50 /ml. Cattle infected by tongue inoculation. C: From [Scott \*et al.\* \(1966\)](#). Titre in calf kidney tissue culture as plaque-forming units (p.f.u.) /ml or /g. Cattle infected by tongue inoculation. Results obtained with seven types and strains of FMD, but the report gives collective values only. D: From [Sellers \*et al.\* \(1969\)](#). Titre in IB-RS-2 tissue culture p.f.u./ml or /g. Cattle infected by indirect contact with infected pigs. E: From [Garland \(1974\)](#). Titre in bovine thyroid tissue culture ID 50/ml or /g. Cattle infected by indirect contact with infected pigs. F: From [Sutmoller and McVicar \(1973\)](#). Titre in calf kidney tissue culture p.f.u./ml or /g or / sample. Cattle infected by intranasal instillation. G: From [Hyslop \(1965\)](#). Remarks as for A above. H: From [Cottral \*et al.\* \(1968\)](#). Remarks as for A above.

\*Physiological data from [Swenson \(1970\)](#); [Sellers \(1971\)](#).

<sup>†</sup>Theoretical calculation, assuming that the daily output is contaminated to the maximum detected level of infectivity.

contaminated by excretions and secretions such as milk, vesicular fluid, saliva, urine or faeces and then handle other animals. Other mechanical means of spread include the use of animal transport vehicles which have not been cleaned and disinfected, and the transport of contaminated milk in bulk milk tankers. The connection and

disconnection of filling pipes of tankers on farms are procedures likely to cause the spread of virus. The generation of infectious aerosols from the exhaust vent of tankers during the filling process might also seem to present a potential mode of spread; this risk, which has been shown experimentally to be remote, could be eliminated by fitting

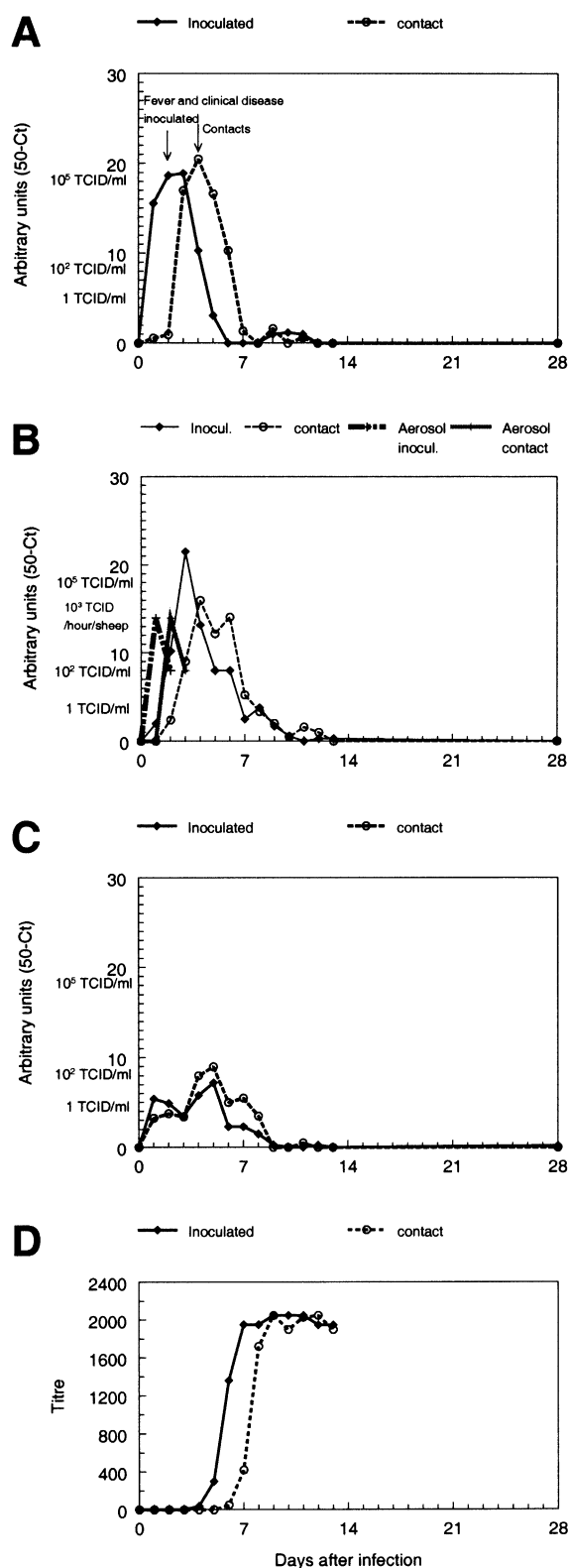


Fig. 1. A–D. FMDV genomes and antibodies in samples from sheep experimentally infected with FMDV O UK 34/2001 virus. Modified from Alexandersen *et al.* (2002c), with the permission of the Society for General Microbiology. (A) FMDV genomes in serum samples

suitable air filters to the vent or directing the exhaust air through the engine (Harper, 1968; Hedger and Dawson, 1970; Sellers, 1971; Donaldson, 1997).

Studies with sheep experimentally infected with the UK 2001 strain showed that viral excretion by inoculated and contact-infected animals occurred in three phases: firstly, a highly infectious period of around 7–8 days; secondly, a period of 1–3 days when trace amounts of viral RNA were recovered in nasal and rectal swabs; and thirdly, a carrier state in 50% of the sheep (Alexandersen *et al.*, 2002c). Similar studies with experimentally infected cattle and pigs showed that airborne viral excretion coincided with the appearance of vesicular lesions and occurred within the viraemic phase. Viral RNA was recovered in nasal swabs from inoculated animals soon after they developed a viraemia and probably reflected early production and excretion of virus. The detection of infectivity (Garland, 1974) and more recently of viral RNA (Alexandersen *et al.*, 2003b) in nasal swabs from contact animals up to 3 days before they showed signs of infection, and in animals after the viraemic phase, probably represented background environmental virus that had been inhaled and trapped in the respiratory tract. The pattern of excretion by pigs was similar to that in cattle, but the amounts of viral RNA recovered in the blood and breath were higher (Alexandersen *et al.*, 2003b).

from sheep. The average level in each group is shown in relation to the days after the start of the experiment. Signals are expressed as 50-Ct and for certain levels the corresponding values for TCID<sub>50</sub>-equivalents/ml are shown (converted as described by Oleksiewicz *et al.* (2001) and Alexandersen *et al.* (2001)). The start of fever (>40 °C) in the inoculated (*n* = 6) and contact-infected (*n* = 4) groups is also indicated. (B) FMDV genomes in nasal swab samples from sheep. The average level in each group (inoculated sheep *n* = 6 and contact sheep *n* = 4) is shown in relation to the day after the start of the experiment. Signals are expressed as 50-Ct and for certain levels the corresponding values for TCID<sub>50</sub>-equivalents/ml are indicated. The corresponding levels of airborne virus excreted from the sheep are also indicated as the amount of airborne virus excreted per sheep per hour. (C) FMDV genomes in rectal swab samples from sheep. The average level in each group (inoculated sheep *n* = 6 and contact sheep *n* = 4) is shown in relation to the days after the start of the experiment. Signals are expressed as 50-Ct and for certain levels the corresponding values for TCID<sub>50</sub>-equivalents/ml are indicated. (D) Detection of antibodies in sera by liquid-phase-blocking-ELISA. All negative samples have a titre less than 1 in 16 while a titre of more than 1 in 40 is considered positive (Donaldson *et al.*, 1996). Inoculated sheep *n* = 6 and contact sheep *n* = 4.



A sharp decline in viral excretion and load occurs around day 4–5 of clinical disease, when a significant antibody titre can be detected. However, it is important to note that although all secretions and excretions (other than oesophageal–pharyngeal fluid in ruminants) are free of detectable infectivity at 10–14 days post-infection, virus already excreted during the preclinical and acute clinical phases (see [Tables 2–4](#)) can survive in the environment for weeks or even months. Such survival will depend, however, on the nature of the material contaminated (manure, fodder, bedding, footwear, clothing, equipment, vehicles, other fomites etc.) and the ambient conditions (weather, moisture, relative humidity, temperature, pH, etc.). While there have been isolated reports of survival of the virus for extended periods (e.g., on hay for at least 200 days, and in faecal slurry for 6 months ([Hyslop, 1970](#))), few quantitative data are available on the persistence of FMDV in the environment ([Cottral, 1969](#); [Sanson, 1994](#); [Donaldson, 1997](#)). However, in general terms, the environmental factors that favour survival of virus are moist conditions, a neutral pH and low temperature.

#### *The Antibody Response*

Significant titres of circulating antibodies, which can be detected by enzyme-linked immunosorbent assay (ELISA) at around 3–5 days after the first appearance of clinical signs, become high 2–4 days later. Further details are shown in [Fig. 1D](#). The earliest detection of antibodies by virus neutralization assay (see later) is usually 1–2 days later than by the more sensitive ELISA. Coincident with the first detection of antibody there is a progressive clearance of virus from the circulation and a significant reduction from most organs, excretions and secretions. An exception is the pharyngeal region of ruminants, since up to 50% or more animals may become persistently infected and excrete virus in oesophageal–pharyngeal fluid. Viral excretion by carrier animals is intermittent and declines progressively (see below). The antibody response normally remains high for many months after infection, and may still be detectable after several years. However, in pigs, especially in fast-growing young animals, the antibodies may have a half-life as little as 1 week and may remain detectable for only a few months. As mentioned earlier, immunity to FMD is primarily mediated by circulating antibodies, and protection after recovery from infection or after immunization (active or passive) is closely correlated with the titres of circulating antibodies, despite the fact that these

antibodies are ineffective in clearing virus from the pharynx of carrier ruminants ([Brown and Cartwright, 1960](#); [Hess \*et al.\*, 1967](#); [McVicar and Suttmoller, 1974](#); [McVicar and Suttmoller, 1976](#); [Francis and Black, 1983](#); [Francis \*et al.\*, 1983](#); [Black \*et al.\*, 1984](#); [Hamblin \*et al.\*, 1987](#); [McCullough \*et al.\*, 1992](#); [Barnett and Carabin, 2002](#)). Circulating antibodies capable of binding to the surface of virions will facilitate opsonization and uptake by phagocytes located in the liver, spleen and elsewhere, and thus rapidly reduce or prevent viraemia ([McCullough \*et al.\*, 1992](#)). As viraemia is an important phase in the early infection process (see later), its reduction or prevention will have a corresponding effect on acute generalized disease. However, since circulating antibodies will not prevent primary local infection (e.g., at the site of intradermal inoculation, or in the pharynx) it would seem that they prevent disease but not infection ([McVicar and Suttmoller, 1976](#)).

#### *Pathogenesis of Acute Disease*

*Clinical signs and development of lesions.* FMD is characterized by an acute febrile reaction and the formation of vesicles in and around the mouth and on the feet. The resultant pain causes lameness, manifested by foot “flicking”, a tucked-up stance and reluctance to stand or walk, as well as inappetence. On handling, heat and pain may be detected in the feet for 1–2 days before vesicular lesions appear. Lesions are often observed initially as blanched areas, which subsequently develop into vesicles. Vesicles may also be seen on the snout or muzzle, teats, mammary gland, prepuce, vulva and other sites of the skin; they are found most consistently, however, in and around the mouth and on the feet. At post-mortem examination lesions may also be found on the ruminal pillars. Lameness, especially in sheep, may not be a consistent finding in all animals. However, in an infected flock of sheep, careful examination will usually reveal some lame animals, especially during the acute stages of disease. Animals kept on soft bedding are less likely to develop severe foot lesions and show lameness. Clinical disease is usually severe in pigs, and the early signs include acute lameness, reluctance to stand, adoption of a dog-sitting posture, depression, loss of appetite and fever. Lesions of the feet may include the shedding of claws (“thimbling”), and the accessory digits may be affected, as well as pressure points on the knees and hocks ([Fig. 2](#)). In cattle, the clinical signs, which are generally obvious, include the drooling



Fig. 2. FMD generalized lesions in pigs 3–5 days after exposure to pigs inoculated with FMDV O UK 2001. Lesions are evident along the coronary bands, the snout and at the back of the tongue.

of saliva and mouth lesions that are often typical and rather severe (Fig. 3), and sometimes lesions of the feet. In sheep and goats the signs may be severe but are generally much more subtle than in pigs and cattle. In mild cases the lesions are superficial and transient, and heal rapidly (Fig. 4). Serological field surveys and experimental investigations have shown that FMD in small ruminants may be clinically inapparent in a significant proportion of animals (Donaldson and Sellers, 2000). Fluid-filled vesicles, which are readily seen in cattle, are rarely observed in the mouth of sheep and goats, probably because the thinness of the lingual epithelium causes superficial lesions to rupture early, leaving shallow erosions which usually heal within a few days. Lesions in the mouth of large and, to a lesser extent, small ruminants are most often seen on the dental pad and the tongue but may also be seen on the lips, gums, and cheeks, and sometimes on the hard palate. In pigs, mouth lesions, when present, are most often located on the tongue, either far back on the dorsum or as tiny lesions at the tip. Vesicles on the feet of pigs and cattle are most often seen

in the interdigital space, at the bulb of the heel, and along the coronary band. As in sheep and goats, oral lesions in pigs may heal without much exudate or subsequent scarring. Soon after rupture, the base of vesiculated areas becomes covered within a few days by a serofibrinous exudate. The regeneration of epithelia is usually well advanced within 2 weeks. However, there is usually a variable degree of scarring, in particular after the occurrence of severe lesions. The rupture of vesicles, especially on the feet or teats, may predispose the affected areas to secondary infections, which may complicate and prolong the healing processes. The age of lesions can be assessed by examining the stage of their development according to the following established criteria: development of vesicles from days 0 to 2; rupture of vesicles on days 1–3 (initially having fragments of epithelia attached); followed by sharply marginated erosion (days 2–3); with the sharpness lost from day 3; serofibrinous exudation on days 4–6; and the beginning of repair with a marked fibrous tissue margin at 7 or more days (Anonymous, 1986). Severe lesions



Fig. 3. FMD generalized lesions in cattle 6 days after exposure to cattle inoculated with FMDV O UK 2001. Lesions are evident on the tongue, dental pad, gums and feet.

of the coronary bands, as seen especially in pigs, may often lead to a separation of the horn during the acute inflammatory stage. This results in the formation of a ring in the horn that becomes visible below the coronary band approximately 1 week after the first appearance of clinical disease. This ring progresses down the hoof since the horn grows at a rate of approximately 1–2 mm per week (horn growth is fastest in young animals). In pigs, the severe inflammation of the feet during the acute stage may sometimes cause the horn of the hoof to be completely separated and shed (“thimbling”; Fig. 5), resulting in severe lameness. A similar effect may occasionally be seen in sheep and cattle but usually only in cases with severe secondary bacterial infection. FMDV strains may differ in their virulence for different species. For example, the O Taiwan 1997 strain caused severe lesions in pigs, but no cases were seen in ruminants (Dunn and Donaldson, 1997).

The marker for the severe virulence of the O Taiwan 1997 strain for pigs and the absence of virulence for cattle is associated with changes in the 3A gene of the virus (Knowles *et al.*, 2001a). Virulence of FMDV strains may also vary between breeds of animal and sometimes within a breed, probably because of genetic or physiological factors.

Mortality in adult animals is generally low, but it may be high in young animals, including calves and especially lambs and piglets, due to acute myocarditis. Macroscopical examination of the heart in these cases often reveals a soft, flaccid heart with white or greyish stripes (the so-called “tiger heart”) or spots, seen mainly in the left ventricle and interventricular septum. In young animals dying from hyper-acute disease there may be no significant macroscopical lesions in the heart (and an absence of vesicular lesions), but virus can usually be isolated from the myocardium or from





Fig. 4. FMD generalized lesions in sheep 4 days after inoculation with FMDV O UK 2001. Lesions can be observed along the coronary band when the feet are carefully cleaned and the hair clipped to allow close inspection. Superficial lesions are evident on the dental pad and tongue; however, these lesions are superficial, rupture early and heal within a few days.

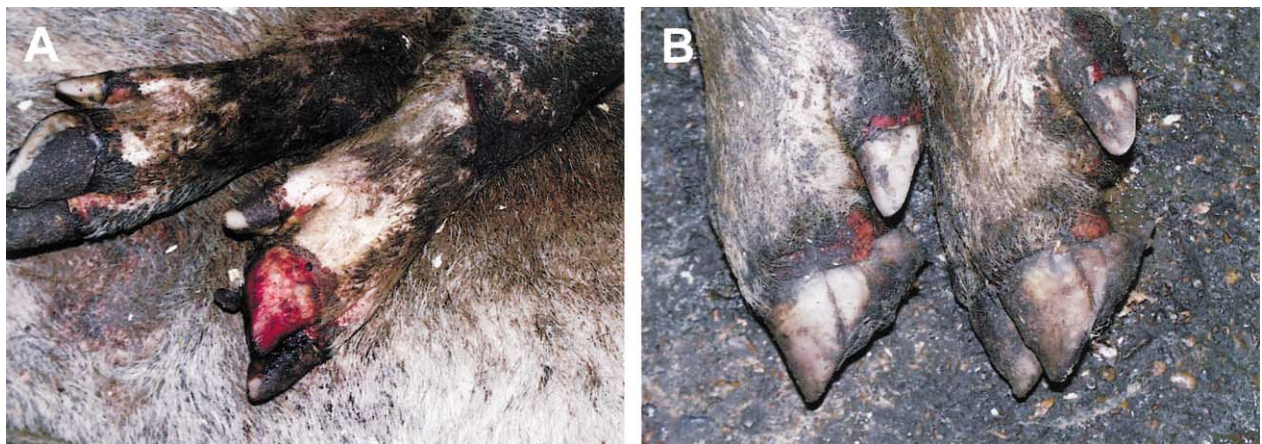


Fig. 5. FMD in pigs from the field during the UK 2001 type O epidemic. (A) The horn is shed from a digit and the exposed surface beneath is observed. (B) Severe lesions of the heel pad area.



the blood, and lesions can be detected by histopathological examination (Donaldson *et al.*, 1984). Occasionally, the skeletal muscles may also be affected. No lesions can be observed in the myocardium or skeletal muscles of older animals and virus appears not to replicate in such sites except in young animals (Alexandersen *et al.* unpublished). The significance of FMD acute myocarditis in young animals in the spread of the disease is not well understood. Possibly there is little or no excretion of virus, since death usually occurs early, before the development of vesicular lesions. However, although in such cases FMDV replicates mainly in the heart, it appears plausible that a significant viraemia occurs (Donaldson *et al.*, 1984); if so, virus may be present in breath, saliva, nasal fluid etc. To our knowledge these possibilities have not been investigated. FMD may also cause abortion in pregnant animals; however, the precipitating factors for the various livestock species have not been determined.

In FMD, skin lacking macroscopical lesions (with [Gailiunas, 1968] or without microscopical lesions) may contain virus, albeit in amounts smaller than those present in visible lesions (Alexandersen *et al.*, 2001). It is possible that scattered cells infected from the circulation are not observed as visible lesions and that virus from such cells is mainly released into the circulation. In contrast, we hypothesize that at sites of trauma or intensive physical stress there may be increased lateral or local spread to additional cells (Platt, 1961), leading to larger foci of infected cells which, together with a physical separation of damaged tissue, can be observed as vesicles. This hypothesis may not entirely explain the numerous visible lesions that occur along the coronary band, as this region would not appear to be under increased external physical stress as compared with other skin nearby. However, the high vascularity of the coronary band in combination with the severe local inflammatory response may lead to cutaneous tension and stress and increased vascular permeability, both of which are likely to contribute to the development of visible vesicular lesions (Platt, 1961).

It is puzzling that no lesions develop on the soft palate or the dorsal part of the pharynx, despite the presence of significant amounts of virus. It may be hypothesized that the infection causes no acute cytopathology in the transitional epithelial cells found in these areas, or that viral cytopathology is restricted to a few cells and is therefore not easily detected, or that because the epithelia of this region are non-cornified the development of

distinguishable lesions is somehow prevented. However, the underlying mechanisms behind this apparent lack of cytopathology are currently unknown.

The first histopathological changes in the cornified, stratified squamous epithelium are ballooning degeneration and increased cytoplasmic eosinophilic staining of the cells in the stratum spinosum, and the onset of intercellular oedema within the dermis. These early lesions (Fig. 6) are detectable only by microscopical examination (Gailiunas, 1968; Yilma, 1980) and, as indicated earlier, apparently normal skin may contain significant amounts of virus (Alexandersen *et al.*, 2001). This early stage may be followed by necrosis and subsequent mononuclear cell and granulocyte infiltration; the lesions, now macroscopically visible, develop further into vesicles by separation of the epithelium from the underlying tissue and filling of the cavity with vesicular fluid (Fig. 6). In some cases the vesicular fluid production may be high and the resulting vesicles large. In other cases the amount of fluid may be limited and the epithelium may undergo necrosis or be torn off by physical trauma without the formation of an obvious vesicle. The variability seen is most likely due to combinations of viral strain virulence, thickness of the affected skin, and husbandry conditions (especially as they affect physical stress on different regions of the skin).

In young animals dying from acute disease, there is lympho-histiocytic myocarditis with hyaline degeneration, necrosis of myocytes and infiltration with mononuclear cells.

*Mechanisms responsible for fever and general depression/dullness, including immune complexes, cytokines and acute phase proteins.* The temporal pattern of FMDV replication and the development of specific lesions and of acute clinical disease, are well described; relatively little is known, however, about the specific mechanisms responsible for the acute clinical signs, such as fever, general depression/dullness and reduced feed intake. As mentioned earlier, FMD is generally severe in pigs and cattle, but the severity of the clinical signs is not always directly related to the severity of the lesions observed. Furthermore, although FMDV and swine vesicular disease virus (SVDV) cause closely similar lesions in pigs, FMDV usually causes much more severe clinical signs. It appears likely that, in addition to the lesions, FMDV infection induces a relatively severe proinflammatory reaction, resulting in fever, general depression/dullness, reduced feed intake, occasional inability to maintain body temperature, and even mortality. These aspects of FMD, although not well

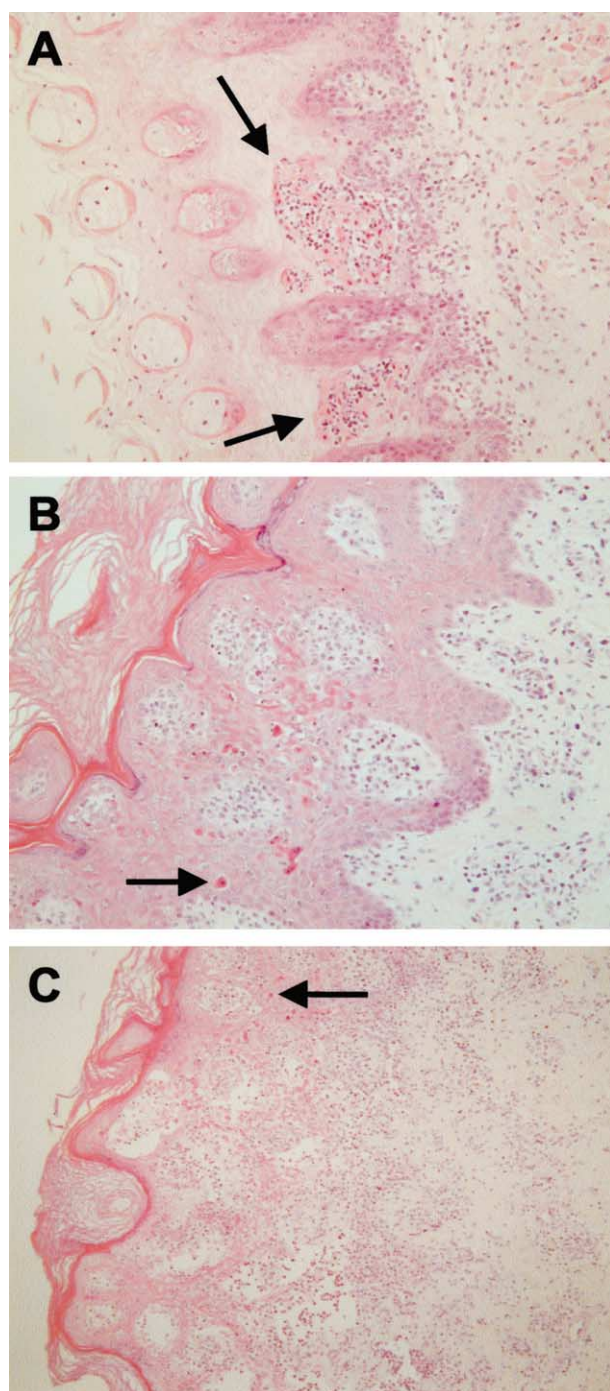


Fig. 6. Formalin-fixed, paraffin wax-embedded sections of tissue from pigs inoculated with FMDV O Taiwan 1997 in the heel pad of a left fore foot. (A) Section of tongue 3 days after inoculation with a low dose of virus. Macroscopically, early vesicular lesions were evident on all four feet and the tongue; however, the area of the tongue shown had no recognizable macroscopical lesions. Two early microscopical lesions (microvesicles) can be observed (arrows) as small areas of swollen cells with an eosinophilic cytoplasm in the stratum spinosum of the epithelium. Such microscopical lesions, which are observed only rarely in sections from macroscopically normal skin of infected animals,

understood, are likely to result from virus–host interactions extending beyond the observed acute cytopathology in virus-infected cells. Factors responsible are likely to include cell death (releasing so-called “danger signals”), virus-antibody immune complex formation, complement activation, and the release of cytokines, prostaglandins and acute phase proteins. Below we summarize what is known, and give preliminary results and hypotheses arising from recent work.

A number of studies indicate that interferons alpha and beta have a role in the host control of FMDV infection and that various isolates of FMDV may differ in their ability to induce an interferon response (Sellers, 1963; Seibold *et al.*, 1964; Cottrel *et al.*, 1966; Fellowes and Suttmoller, 1970; Mohanty and Cottrel, 1971; Richmond, 1971; Sellers *et al.*, 1972, 1973; Kaaden *et al.*, 1973, 1975; McVicar *et al.*, 1973; Amadori and Melegari, 1987; Chinsangaram *et al.*, 1999; Brown *et al.*, 2000). Additional studies suggest that plaque size in FMDV-infected cultures reflects the extent of interferon induction. For example, studies with bovine cells showed that large plaque size and low interferon induction was associated with high infectivity for cattle by the intramuscular route (Cottrel *et al.*, 1966). Similarly, large plaque size in porcine cells was associated with isolates of high virulence for pigs (Sellers *et al.*, 1959; Borgen and Schwobel, 1964). Studies on haptoglobin (Hofner *et al.*, 1994) indicated that this acute phase protein is elevated in FMDV-infected cattle when viraemia and clinical signs become evident, suggesting that the inflammatory response is activated. We performed preliminary studies to examine the role of interferon-gamma in the development of persistent FMDV infection

probably progress rapidly into macroscopical lesions (see B and C). Haematoxylin and eosin (HE).  $\times 200$ . (B) Section of skin from the coronary band of a hind leg at day 3 after inoculation with a larger dose of virus. The tissue was taken at the edge of a macroscopically visible, early vesicular lesion. Outside the lesion, no significant microscopical lesions are observed (top) while at the edge of the lesion (centre and bottom) swollen cells with an eosinophilic cytoplasm are seen (arrow) in the stratum spinosum, probably representing early acute cytopathological changes. HE.  $\times 200$ . (C) The same section of skin (from the coronary band of a hind leg at day 3 after inoculation) as that shown in Fig. 3B. The micrograph, taken at a lower magnification, includes the edge of the macroscopically visible lesion. At the top, early cellular cytopathology is evident (swollen cells with an eosinophilic cytoplasm; arrow); and below, cytopathology, cell infiltration and vesicle formation are obvious. HE.  $\times 100$ .

in cattle (Zhang *et al.*, 2002) and have now expanded these studies to include acute FMDV infection in pigs. In these studies, examination of the mRNA levels of several antiviral and proinflammatory cytokines in peripheral blood mononuclear cells (PBMCs) and tissues indicated that FMDV infection resulted in a complex interaction of both the induction and the degradation of mRNAs, and in increased concentrations of circulating haptoglobin (Murphy, Ahmed, Zhang and Alexandersen, unpublished). Further investigation would seem merited.

### **Persistent Infection with FMDV: The Importance of the Carrier Problem and Mechanisms Involved in Establishment of Persistent Infection**

The notion that FMDV can cause persistent infection (i.e., produce carrier animals) was initially based on field outbreaks, but remained conjectural until van Bekkum *et al.* (1959a,b) showed the continuing presence of infectious virus in the “saliva” (actually the oesophageal-pharyngeal [OP]) fluid) of a proportion of convalescent cattle for many weeks after infection. These findings stimulated further studies during the next 10–15 years (see Salt, 1998; Alexandersen *et al.*, 2002b), demonstrating that persistent infection (defined as the presence of detectable virus for at least 28 days after infection [Sutmoller and Gaggero, 1965; Sutmoller *et al.*, 1968]) could be found in a proportion of infected cattle, sheep and goats. Pigs, however, cleared FMDV infection in 3–4 weeks and so did not become carriers. A single report claimed that persistent FMDV infection occurred in pigs and it has also been hypothesized that, in pigs, FMDV infects the alveolar macrophage, a likely candidate for supporting the carrier state (Baxt and Mason, 1995; Mezcencio *et al.*, 1999; Rigden *et al.*, 2000). However, many studies, including our own based on isolation of virus in BTY cells and on quantitative RT-PCR, have consistently failed to show that FMDV persists in pigs for more than 3–4 weeks.

It is well established that the African buffalo can carry the virus for up to 5 years or more (Hedger, 1972; Thomson *et al.*, 1984; Condry *et al.*, 1985; Hedger and Condry, 1985). Other cloven-hoofed wildlife species, including deer and impala, which may become acutely infected, either do not become carriers or do so for only a relatively short period and so are unlikely to play an important epidemiological role as carriers (McVicar and Sutmoller, 1969a; Hedger *et al.*, 1972;

McVicar *et al.*, 1974; Gibbs *et al.*, 1975; Thomson *et al.*, 1984; Bastos *et al.*, 2000).

Current knowledge can be summarized as follows: some ruminant animals exposed to FMDV become carriers, irrespective of whether they are fully susceptible or immune (i.e., protected from disease as a result of vaccination or recovery from infection). The percentage of animals that become carriers under experimental conditions is variable but averages around 50%. The infectivity titre of virus in OP samples from carriers is usually low (*c.* 10–100 TCID<sub>50</sub>/ml); excretion is also intermittent and the titre declines over time. Both the animal species and strain of virus appear to be determinants in the development and persistence of the carrier state. The maximum reported duration of the carrier state in different species is as follows: cattle, 3.5 years; sheep, 9 months; goat, 4 months; African buffalo, 5 years; water buffalo, 2 months. It should be noted that information on the water buffalo is very limited, only a few such animals having been examined; however, a single experimental study from Egypt suggested a carrier state lasting at least 6 weeks (Moussa *et al.*, 1979).

### *Definition of the Pharynx as the Site of Persistence*

The “probang” (cup probang or probang cup) was used in early studies by van Bekkum *et al.* (1959a,b) for taking samples of OP fluid for the detection of FMDV in carrier animals. It was later slightly modified (Sutmoller and Gaggero, 1965; Burrows, 1966a) to a form essentially identical with that used today.

It is uncertain whether the virus in probang samples is mainly cell-free (possibly existing as an immune complex with antibody), or whether it is initially cell-associated (or located in “cytoplasmic blebs” [Yilma *et al.*, 1978; Donn *et al.*, 1995]) and subsequently released during processing before being bound to antibodies. However, virus is commonly found in OP fluids containing cellular material, while samples without cellular material are usually negative. Furthermore, the amount of virus in OP fluid samples, even those taken under optimal conditions, is generally low. The recovery of virus from carriers can be maximized by treating samples with fluorocarbon (e.g., freon) to remove any blocking antibodies and cellular membranes. However, BTY cells are the most sensitive cells for isolating FMDV and the increase in the amount of virus resulting from freon treatment when BTY cells are used for the assay is generally less marked than with other cell systems (Brown and Cartwright, 1960; Burrows, 1966a; Sutmoller and



Cottral, 1967; Suttmoller and McVicar, 1968; McVicar and Suttmoller, 1969a; Donn, 1993). The RT-PCR assay is another highly sensitive and specific method for detecting positive OP samples. Such quantitative assays have been developed and automated (Alexandersen *et al.*, 2001, 2002c; Oleksiewicz *et al.*, 2001; Reid *et al.*, 2001b, 2002; Hearps *et al.*, 2002) and preliminary testing has produced promising results. These new methods should make it possible to identify the cellular origin and nature of the cell or membrane association of virus in the probang samples.

In-situ hybridization studies have shown that certain epithelial cells of the pharynx in carrier cattle are positive for FMDV genomic material (Woodbury *et al.*, 1995; Murphy *et al.*, 1999; Zhang and Kitching, 2000, 2001). When such cells are cultured they remain virus-positive (Mohanty and Cottral, 1971; Zhang *et al.*, 2002). Interestingly, when non-cornified, dorsal, soft palate tissue was placed in culture it was immediately susceptible and supported productive FMDV replication. By contrast, the cornified floor of the pharynx became susceptible only after 2 days in culture, coincident with the shedding of the cornified (dead) cell layer in culture (Williams and Burrows, 1972). Thus, it seems highly likely that the target region for persistent infection in cattle is the pharynx and, more specifically, the dorsal soft palate and the dorsal part of the pharyngeal roof located above the soft palate. These pharyngeal sites are apparently the same as those that play a role in the early (acute) phase of FMDV infection and it is noteworthy that no significant histopathological changes have been reported to occur in this area, even in acute infection (Salt, 1998; Alexandersen *et al.*, 2001, 2002b). It is also interesting that in pigs infected by contact exposure FMDV has been found in relatively high concentrations in soft palate, tonsil and pharynx in early infection (Alexandersen *et al.*, 2001; Oleksiewicz *et al.*, 2001). However, at 3 weeks after infection there was no detectable virus or viral genome (as indicated by tissue sample analysis) in this species (Alexandersen *et al.*, unpublished). By contrast, viral infectivity and genome material could be detected in OP fluid and samples of pharyngeal tissue from carrier ruminants for more than 4 weeks, although the levels were low compared with those in samples taken during acute infection (Burrows, 1966a, 1968b; Suttmoller and McVicar, 1968; McVicar and Suttmoller, 1969b; Kaaden *et al.*, 1975; Alexandersen *et al.*, 2002b; Zhang and Alexandersen, unpublished).

#### *Evidence of Persistence at Sites Other Than the Pharynx*

This matter has not been studied intensively. However, the mammary gland and testicles may contain virus for several weeks after infection and the pituitary, pancreas and thyroid have been suggested as additional sites of persistence (Jones, 1965; Scott *et al.*, 1965; Cottral *et al.*, 1968; Sellers *et al.*, 1968, 1969; Burrows *et al.*, 1971; McVicar and Suttmoller, 1971; de Leeuw *et al.*, 1978). Recent data indicate that organs such as the kidneys and lymph nodes are not likely to be sites of persistence; FMDV RNA sequences have been found in the mandibular lymph node in carrier cattle (Zhang and Alexandersen, unpublished), but this was probably due to the drainage of virus from the pharynx. Based on dot blot analysis, it has been suggested that FMDV RNA may be associated with PBMCs, even during persistent infection (Bergmann *et al.*, 1996). However, using quantitative RT-PCR, we detected relatively low concentrations of FMDV RNA in PBMCs from infected pigs or cattle during the first 5–7 days of the acute stage of the infection, but not later. We conclude that during the acute phase, virus circulates mainly as free virus in the bloodstream, but when antibodies are produced they form complexes with the virus; these complexes are cleared within a few days and virus is then no longer detectable in the blood (Hess *et al.*, 1967; Suttmoller *et al.*, 1968; Cottral and Bachrach, 1968; McCullough *et al.*, 1992; Alexandersen *et al.*, 2002c and unpublished).

#### *Evidence of Transmission from Carrier Animals*

As mentioned earlier, field outbreaks (e.g., the recrudescence outbreaks in the UK 1922–1924 and other incidents such as those in Denmark 1883–94 [Hedger and Stubbins, 1971] and 1982–83 [E. Stougaard, personal communication]) provided circumstantial evidence that carrier animals play a role in the epidemiology of FMD, a theory later strengthened by the demonstration of infectious FMDV in OP fluid samples. Reports of transmission from carrier animals (African buffalo) to cattle in the Zimbabwean outbreaks of 1989 (SAT 1) and 1991 (SAT 3), which were supported by laboratory characterization of the causal viruses, provided stronger evidence for a role of carriers in recrudescence outbreaks (Dawe *et al.*, 1994a). Sexual contact may have played a role in the experimental transmission from carrier African buffalo to cattle (Thomson *et al.*, 1984; Hedger and Condry, 1985; Dawe *et al.*, 1994b; Bastos *et al.*, 1999, 2000). Outbreaks of the SAT 2 serotype in Zimbabwe in 1989 and 1991 indicated transmission from carrier



cattle to uninfected cattle (N.J. Knowles, unpublished observations, cited by [Salt, 1998](#)). Thus, current evidence indicates that the risk of transmission from carrier African buffalo to cattle is significant and that transmission from carrier cattle to uninfected cattle, at least of serotype SAT 2, cannot be excluded.

The epidemiological significance of the carrier state in cattle and sheep remains controversial. Unequivocally, FMD will result when virus-positive OP fluid from carrier animals is *injected* into cattle and pigs ([Van Bekkum \*et al.\*, 1959a,b](#); [Kaaden \*et al.\*, 1975](#)). Therefore, carrier virus is fully infectious and consequently the carrier state is associated with at the least a theoretical risk of spreading the disease. However, attempts to demonstrate transmission from known carrier cattle or sheep to susceptible animals held in close, direct contact under controlled experimental conditions have all failed. Moreover, studies in which dexamethasone treatment or co-infection with bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) was used, with the aim of weakening the immune system and increasing virus levels and excretion, failed to achieve transmission from carriers to susceptible in-contact cattle or pigs ([McVicar and Suttmoller, 1969b](#); [McVicar \*et al.\*, 1976](#)). However, as dexamethasone treatment temporarily “removes” FMDV from the pharyngeal region of carrier animals ([Ilott \*et al.\*, 1997](#)), these studies did not provide convincing evidence against the possibility of transmission from carrier animals under field conditions. Physiological state may significantly affect interaction between animals. Thus, for example, heightened sexual activity in the male camel may cause protrusion of the soft palate from the mouth ([Reece and Chawla, 2001](#)). Sexual contact, as indicated for African buffalo (see above), may also increase the risk of transmission from carrier cattle and sheep. Since the average duration of carriage in sheep is considerably shorter than that in cattle, carrier sheep may pose a much lower risk. On the other hand, FMD often spreads slowly in sheep flocks, causing only mild disease; it may therefore go unnoticed through several cycles of infection, maintaining infectivity for a long period. Therefore, acutely infected sheep, if unnoticed, and sheep in the pre-clinical phase of the infection may constitute a significant risk of “silent” spread of FMD, as demonstrated by the UK 2001 epidemic. Similarly, “silent” low level circulation of infection may also occur in pig farms if vaccination is introduced after the infection has become established ([Kitching and Alexandersen, 2002](#)).

Due to practical constraints, experimental studies on the possible transmission of infection from carrier to susceptible animals have necessarily been carried out with small numbers of animals and comparatively few serotypes and strains of virus. The negative results obtained do not prove that the possibility can be excluded. Such transmission may be a rare event, requiring certain trigger factors not easily or often reproduced under experimental conditions, e.g., the chance encounter of a high excretor of virus with a highly susceptible (possibly immunosuppressed) recipient. While the event certainly appears to be rare, the risk is not zero. The consequences of FMD are so dire, especially for countries with an unprotected livestock population, that the possibility of spread by carrier animals cannot be dismissed.

#### *Carriers Under Field Conditions*

The prevalence rate of carriers depends on the species, the incidence of disease (or infection) and the immune status of the population. The prevalence of carrier African buffalo can be as high as 50-70% under free-living conditions ([Hedger, 1972](#); [Condy \*et al.\*, 1985](#)). In endemic areas, the prevalence rate in domesticated livestock may also be relatively high. For example, a study in Botswana showed that around 20% of the cattle were carriers, and a survey in Asiatic Turkey revealed a corresponding figure of 15–20% for cattle and sheep ([Hedger, 1968](#); [Gurhan \*et al.\*, 1993](#)). A carrier prevalence rate of 50% in cattle in Brazil was found 6 months after a vaccine breakdown ([Suttmoller and Gaggero, 1965](#)). [Anderson \*et al.\* \(1976\)](#) suggested that effective vaccination, although not preventing the development of the carrier state in an animal exposed to live virus, reduced the prevalence of carriers in the field by decreasing the overall weight of challenge of the animals. A useful strategy might therefore be to maintain a high level of vaccination coverage over a long period, thereby reducing the prevalence of residual carriers and the potential risk of effective disease transmission. This should be accompanied by intensive clinical surveillance of susceptible (non-vaccinated) species, which, in most situations, will be the pig population. These actions could be strengthened by surveys for carrier animals by means of tests for antibodies to the non-structural (NS) proteins of FMDV, combined with probang sampling, bearing in mind that both methods have practical and statistical limitations ([Hedger, 1971](#); [Hedger and Stubbins, 1971](#); [Mackay, 1998](#)).

### *Possible Mechanisms Behind Persistence in the Pharynx*

The factors determining the establishment of persistent FMDV infection are poorly understood. However, the carrier state can develop either after the acute stage of disease or in the absence of any clinical disease, for instance in vaccinated or recovered animals exposed to live virus. Nevertheless, in both situations the systemic replication of virus is controlled or restricted, either immediately by the vaccine-induced immunity, or a few days after infection by the antibody then produced. However, pharyngeal sites have the ability to support viral replication in the presence of circulating antibodies (Hedger, 1970; McVicar and Suttmoller, 1976).

The reason why the pharyngeal region in the pig does not become persistently infected is currently unknown but is worthy of further investigation. The much smaller (*c.* 100-fold; Alexandersen, unpublished) area of specialized epithelium on the dorsal soft palate of pigs than cattle may be relevant. It may also be hypothesized that this is a reason why ruminants are much more susceptible than pigs to airborne transmission.

Based on available information, and on a comparison with other persistent viral infections, Alexandersen *et al.* (2002b) postulated that one of two mechanisms may play a role in the development of persistent FMDV infection in the pharynx of ruminants. The first proposal is that virus infects the cells of the immune system or other immunologically privileged sites, for example the epithelium of secretory or excretory organs, and thus evades the host response. In-situ hybridization data indicate that this is not likely to be a major factor in FMD (Woodbury *et al.*, 1995; Murphy *et al.*, 1999; Zhang and Kitching, 2000, 2001), although the possibility cannot be excluded that cells associated with FMDV persistence in the pharyngeal region are immunologically privileged. This may be an inherent property of these highly specialized epithelial cells (see below) or it may be caused by virus-induced MHC class I “down-regulation” (Sanz-Parra *et al.*, 1998). There is some evidence to suggest that immune complexes containing FMD virus and possibly IgA, together with an Fc receptor-mediated uptake into cells of the immune system, may be an alternative mechanism for interfering with immune clearance (McCullough *et al.*, 1992; Salt, 1993, 1998; Baxt and Mason, 1995; Salt *et al.*, 1996; Ilott *et al.*, 1997). However, in in-vivo studies we have not observed any evidence for significant accumulation of FMD virus in macrophages/monocytes; these cells therefore do not appear

to play a conspicuous role in FMDV replication and persistence. The second proposal, which is an integral part of our working hypothesis, is that in contrast to the normally cytolytic, acute infection, the virus somehow exploits the host response to provide an intracellular milieu favourable for long-term persistence of FMDV. An unknown factor in the development of persistently infected cells is the potential activation of a CD8 T-cell response (Childerstone *et al.*, 1999) which, if capable of recognizing infected cells, should prevent the establishment of persistent infection. The virus would not cause immediate cytolysis of all target cells, and would be able either to suppress CD8 T-cell activation or to abolish recognition of infected cells, for example by down-regulation of MHC class I expression, as mentioned above. Evidence for such mechanisms will require more detailed studies. Recently, it has been suggested that the immune system may “switch off” CD8 T-cells relatively early in infection, even if infection is not fully cleared (Badovinac *et al.*, 2002; Harty and Badovinac, 2002). Consequently, if virus can remain infectious for long enough in the body, say for more than a few weeks, persistence may be facilitated. Our own preliminary studies indicate that even in infected cattle that do not eventually become carriers, infectious virus is present in the pharynx for several weeks, with an apparent half-life much longer than that of virus in non-pharyngeal samples. This virus might be a source of “re-infection” of the pharyngeal epithelial cells at a time when the CD8 T-cell response is diminished or absent. This theory would fit with our preliminary studies, which suggest that when infected cattle are re-exposed to live virus from acutely infected contact cattle they may have an increased probability of becoming carriers (Alexandersen and Zhang, unpublished).

We hypothesize that FMDV exploits the second mechanism, i.e., establishment of an intracellular milieu favourable for persistence; furthermore, we propose that cytokines may play a role in the cell-to-cell signalling cascade that regulates the delicate balance between acute viral cytopathology, immune clearance and survival of virus, which may ultimately lead to the establishment of persistent FMDV infection (Alexandersen *et al.*, 2002b). Interferons (alpha, beta and gamma) are the cytokines likely to play a role in FMD, as well as certain interleukins and perhaps tumour necrosis factor alpha (TNF- $\alpha$ ). Studies are needed on specific cytokines to investigate whether or not they are induced or suppressed in FMDV infection.

The findings should be related to viral replication, disease progression, and the development of persistent infection *in vivo*, to reveal whether these cytokines form part of the mechanism of persistence. The results should then provide insights relevant to the development of better vaccines or of diagnostic assays specific for FMDV persistence. Such developments might even lead to a cure for persistent infection and contribute to the ultimate epidemiological challenge—the elimination of FMD.

What is the explanation for the strong predilection of FMDV for pharyngeal epithelia? As mentioned previously, the epithelial cells at these sites, i.e., the dorsal part of the soft palate and the ceiling of the pharynx just above the soft palate, are highly specialized and form, in contrast to most of the surrounding epithelia, a non-cornified, stratified, squamous epithelium. The special feature of this area is that there is no layer of dead cells on the surface of the epithelia, and the epithelia are relatively thin (Alexandersen *et al.*, 2002b). It would seem likely that such epithelia, if they possessed the appropriate receptors (see below), would facilitate the entry of virus via the pharynx. Other epithelia, including that of the ventral soft palate, are covered by a layer of dead cells (more or less cornified) and are much more likely to be infected “from within”, i.e., haematogenously (Alexandersen *et al.*, 2002b). In addition to facilitating entry of the virus, the epithelium of the dorsal soft palate and dorsal pharynx might also play a significant role in viral persistence. FMDV infection is usually considered highly cytolytic, but the presence of virus in epithelial cells of the soft palate in the absence of significant lesions or cytopathogenic effects suggests that the establishment of persistent infection may depend in part on the type of target cells (Zhang and Kitching, 2000, 2001; Alexandersen *et al.*, 2002b). These cells may provide an entry point for the virus or for virus-containing immune complexes from the OP fluid and also a favourable site for persistent, low-level replication of virus. In contrast, cornified epithelia, which are mainly responsible for viral amplification during acute infection, cease to be infected when antibodies are present, because antibody-virus complexes are efficiently removed from the circulation.

### Receptors

The mechanism by which FMDV virions initiate infection is thought to be based on the attachment of the RGD loop of VP1 on the viral capsid to host integrins on the surface of target cells (McKenna

*et al.*, 1995; Rieder *et al.*, 1996). In certain cell cultures the interaction between virus and cells is different and an altered or selected stretch of VP3 binds to heparin-like moieties on the cell culture surface (Sa-Carvalho *et al.*, 1997; Fry *et al.*, 1999). In cattle, the receptor is thought to be either the integrin alphavbeta3 or alphavbeta5/1 (or both), or perhaps more likely the alphavbeta6 integrin (Rieder *et al.*, 1994, 1996; Jackson *et al.*, 1997, 2000a, b, 2002; Sa-Carvalho *et al.*, 1997; Neff *et al.*, 1998, 2000). The possible relevance of the FMDV receptors to infection and persistence is discussed above.

### Survival of FMDV

FMDV is moderately stable but can readily be inactivated by appropriate disinfectants and heat. In general, most strains are stable within the pH range 7.0–8.5, especially at lower temperatures, but increasingly labile at pH values outside that range (Bachrach *et al.*, 1957; Bachrach, 1968). FMDV is resistant to detergents and organic solvents such as ether and chloroform. The acidity produced in carcass meat during rigor mortis in cattle will inactivate the virus. Such acidity is variable in pig meat and has not been examined in detail for sheep meat. Furthermore, the pH in bone marrow, lymph nodes and certain organs and offal does not decline during rigor mortis; virus can therefore be found in such material (especially if refrigerated or frozen) for an extended period of time, and may cause new outbreaks if fed to livestock as unheated waste food (Donaldson, 1987).

Airborne virus is stable at humidities above 55–60% and drying will inactivate most but not all of the virus. The drying of fluids or organic material containing virus will also inactivate a relatively large proportion, but surviving virus may be more stable after drying, thereby creating a “tail” of infectivity. Sunlight has little or no direct effect on infectivity; any loss is indirect and occurs mainly through the effects of drying and temperature (Donaldson, 1987; Donaldson and Alexandersen, 2003). Survival of the virus in the environment will depend on the nature of the material (e.g., desquamated epithelium, secretions, excretions), the initial concentration of virus in the material, the strain of virus, the humidity, the pH and the temperature, and will therefore be highly variable under field conditions. Typically, the kinetic curve for the decay of FMDV infectivity is biphasic, i.e., an initial steep decay curve is followed by a prolonged, shallow tail. Residual virus may be remarkably resistant, especially in the presence of

high concentrations of organic material. Examples of reported survival times of FMDV are as follows: up to 20 weeks on hay or straw; up to 4 weeks on cow's hair at 18–20 °C; up to 14 days in dry faeces; up to 39 days in urine; up to 6 months in slurry in winter; 3 days on soil in summer and up to 28 days in autumn. In regard to epidemiological significance, however, the important criterion is whether at the time of exposure of an animal there is sufficient residual infectivity in the material or environment to initiate infection. For further information on the survival of FMDV under various conditions readers are directed to the following references: [Bachrach, 1968](#); [Cottrell, 1969](#); [Donaldson, 1987](#); [McColl \*et al.\*, 1995](#). It should be noted that most of the data are difficult to compare due to differences in the experimental procedures and assay methods used. Thus, the time period for which virus will survive in the environment is difficult to predict, and re-stocking after an outbreak has to be done with care, only after thorough disinfection of the premises, and preferably with the initial introduction and monitoring of sentinel animals.

### Spread of Infection and Disease Control

As mentioned earlier, the most common methods of spread of FMD are (1) the movement of infected animals and the direct contact between the infected and susceptible animals, (2) the feeding of contaminated animal products to susceptible livestock, particularly pigs, and (3) indirect contact, i.e., contact with virus from infected animals transported mechanically by persons, livestock vehicles, fomites, or possibly by wild animals and birds. Transmission by all of these routes can, in principle, be prevented by the efficient application of disease control measures. Another mechanism of spread, which is uncontrollable, is the carriage of virus by the wind. As discussed, this form of spread is not uncommon over short distances but only rarely occurs over long distances. It can, however, result in spread over considerable distances, in particular when, under favourable climatic conditions, large plumes of virus generated by large pig units spread downwind to cattle. When long-distance spread occurs the consequences can be dramatic ([Anonymous, 1969](#); [Gloster \*et al.\*, 1981, 1982](#); [Donaldson \*et al.\*, 1982b](#)). Models to analyse and predict the risk of airborne spread of FMDV were used successfully in the UK in 1981 and 2001, and in Italy in 1993 ([Gloster \*et al.\*, 1981, 1982](#); [Donaldson \*et al.\*, 1982b](#); [Maragon \*et al.\*, 1994](#); [Alexandersen \*et al.\*, 2003a](#)).

Extreme measures are required to eradicate FMD and if they are not rapidly and effectively applied there is a high probability that outbreaks will reach epidemic proportions. The objective in eradication is to stop transmission by reducing as quickly as possible the amount of virus emitted from infected premises (IPs) and dangerous contact premises (DCs) and ultimately to eliminate the virus. The methods applied are based on knowledge of the epidemiology of the disease and require the earliest possible identification and stamping out of affected and in-contact cloven-hoofed livestock on IPs, tracing and stamping out on DCs, carcass disposal, movement restrictions and disinfection. Experience has shown that total stamping out is required to ensure the elimination of any persistently infected ("carrier") animals ([Hedger and Stubbins, 1971](#); [Alexandersen \*et al.\*, 2002b](#)). These strategies have been demonstrated to achieve their objectives under varying conditions in many countries. During the UK 2001 epidemic, mathematical models played a major part in influencing disease control strategy; however, they had not been used previously under operational conditions ([Ferguson \*et al.\*, 2001](#)) and the assumptions behind them as well as the justification for the use of such average-based, mathematically-derived, centrally-controlled procedures and their actual impact on efficient disease management remain highly contentious. It is still an open question as to whether the novel control policies had a positive or negative effect on the course of the epidemic, and whether fewer animals might have been slaughtered had historical methods of eradicating FMD been applied. It was not possible to apply conventional methods at the start of the epidemic, as the available manpower resources were quickly overwhelmed. However, the outcome might have been very different had conventional control measures been applied when sufficient personnel had been recruited.

### Diagnosis

#### *Methods for Diagnosis*

Definitive diagnosis of FMD must be carried out at specialized laboratories. The earlier use of the complement fixation test has largely been supplanted by ELISAs due to their sensitivity, specificity and ability to deal with large number of samples. Laboratory diagnosis is usually made by ELISA detection of specific FMDV antigens in epithelial tissue suspensions, often accompanied by concurrent cell culture isolation and the application of



ELISA to any samples showing a cytopathogenic effect (Hamblin *et al.*, 1984; Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988; Ferris *et al.*, 1988). These tests are used to confirm the clinical diagnosis and to identify the FMD serotype. Recently, RT-PCR assays have been developed for the diagnosis of FMDV infection. Although various procedures for conventional RT-PCR have been published, none seems to be of sufficient sensitivity, specificity and robustness for diagnostic work, unless supported by the other techniques (House and Meyer, 1993; Reid *et al.*, 1998, 1999; Moss and Haas, 1999). RT-PCR assays for the serotyping of FMDV have been published, but the procedures are very labour intensive (Rodriguez *et al.*, 1992; Locher *et al.*, 1995; Vangrysperre and De Clercq, 1996; Callens and De Clercq, 1997; Reid *et al.*, 1998, 1999; Suryanarayana *et al.*, 1999). Alexandersen *et al.* (2000) developed an RT-PCR ELISA of increased sensitivity and, furthermore, included a novel SNAP (Simple And Aqueous Phase) hybridization step to obtain optimal specificity combined with speed and ease of use. Both conventional PCR techniques and the novel method described can be employed to provide serotype-specific results (Vangrysperre and De Clercq, 1996; Callens and De Clercq, 1997; Reid *et al.*, 1999; Alexandersen *et al.*, 2000). However, although this test appears to have a high sensitivity and specificity, the number of samples that can be analysed simultaneously is too limited for use in an epidemic. More recently, we evaluated fluorogenic “real-time” (5′-nuclease probe-based) RT-PCR methodology as an FMD diagnostic tool. This method combined the total RNA extraction and RT procedures of conventional RT-PCR with PCR amplification by means of a fluorogenic probe (Alexandersen and Reid, patent pending) in real-time PCR equipment. This method had a high sensitivity and specificity for the detection of FMDV genomes of all seven serotypes; its sensitivity was 100% for samples submitted from the UK 2001 epidemic (Reid *et al.*, 2001b, 2002). The assay has been used on a large number of tissue samples, serum samples, swab samples and tissue culture supernatants and more recently on probang samples, all with encouraging results. The studies have been extended by evaluating automated procedures for the nucleic acid extraction, RT and PCR amplification stages to increase the throughput of samples and reduce the time required for laboratory diagnosis. The results obtained are described in detail elsewhere (Reid *et al.*, 2003). In addition, this assay has been tested on a portable platform, the Cepheid SmartCycler, to evaluate the speed and accuracy of the assay for

potential portable, near-site diagnosis (Hearps *et al.*, 2002).

Many of the tests traditionally used for FMD diagnosis were developed at the OIE/FAO World Reference Laboratory (WRL) for FMD at Pirbright and are described in the OIE Manual of Standards for Diagnostic Tests and Vaccines, 2000 edition.

*Tests for antigen/virus/genome.* The current techniques used for FMD diagnosis are highly sensitive and specific. The type of sample recommended for testing is based on knowledge of the amount of virus present in various tissues, excretions and secretions, as mentioned earlier. For many of the tests, especially the ELISA methods, vesicular epithelium or vesicular fluid is preferred, as these materials contain high titres of FMDV. The more sensitive methods, such as cell culture and RT-PCR, are appropriate for samples likely to contain smaller amounts of infectivity or viral RNA, e.g., blood, swabs, milk, probang samples, tissues and faeces.

Given a satisfactory sample, a positive result for FMD can be obtained in 3–4 h by an antigen ELISA, which also will identify the serotype of the virus present (Hamblin *et al.*, 1984; Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988; Ferris *et al.*, 1988, 1990). However, less satisfactory samples may yield weak, inconclusive or negative results, and small amounts of virus must be amplified in cell cultures. Depending upon the amount of virus present, two 48 h passages of each test inoculum may be required before a final result can be declared. The antigen ELISA detects *c.* 70–80% of positive samples (i.e., samples subsequently being positive in cell culture) submitted annually to the WRL from outside the UK. During the UK 2001 epidemic, *c.* 90% of positive samples from cattle and pigs were detected by the ELISA, probably due to high quality of the samples and the short transport time between the field and the laboratory. However, for samples from sheep the percentage was lower, probably due to the difficulty of finding adequate amounts of suitable lesion epithelium. Consequently, the specificity of a sample directly yielding a positive ELISA result is high, but a negative sample requires further examination in highly susceptible cell cultures. In the WRL, samples are routinely inoculated on both primary bovine thyroid (BTY) cells (Snowdon, 1966) and an established line of pig kidney cells (IB-RS-2 cells) (De Castro, 1964; De Castro and Pisani, 1964). Cultures showing a cytopathogenic effect are confirmed by antigen ELISA of the cell culture supernatants. For most FMDV strains the BTY cell system is about 10 times more sensitive than other cultures (Snowdon, 1966; Burrows *et al.*, 1981); however, certain pig-adapted

strains, e.g., the O Taiwan 1997 strain (Dunn and Donaldson, 1997), grow more easily in IB-RS-2 cells. Virus isolation in BTY and IB-RS cell cultures essentially detects all positive samples with more than 1–5 infectious units per ml or per 0.1 g. However, if poor specimens are submitted, or if the transport conditions are less than optimal, a small proportion of samples may give negative results for infectivity but positive results by ELISA or RT-PCR. This was not seen in UK samples in 2001, but occurs not infrequently in samples submitted to the WRL from abroad.

As mentioned above, real-time RT-PCR methods developed at Pirbright are as sensitive as the combined ELISA/virus isolation system, detecting essentially the same positive samples as those detected by virus isolation, with high sensitivity and specificity, and providing a definitive result on the day of sample receipt. With experimental samples the sensitivity of RT-PCR is higher than that of virus isolation (*c.* 0.1 TCID<sub>50</sub>/ml). Investigation of suspected cases could be further accelerated by testing on or near the farm. However, this type of testing, especially in a portable format, will require further validation (Callahan *et al.*, 2002; Hearps *et al.*, 2002). The current capacity at Pirbright for automated nucleic acid extraction, robotic cDNA conversion and PCR is currently around 60–90 samples/day, but this could be increased if necessary.

In addition to the methods mentioned above, “pen-side” detection (of antigen) methods are under development, based essentially on the same principle as the antigen ELISA described above, but with a pan-serotype monoclonal antibody in a format adapted for field use (Reid *et al.*, 2001a). Laboratory trials indicate that the sensitivity is equal to, or probably greater than, the traditional antigen ELISA. Field validation of such tests is urgently needed. As for the laboratory based antigen-ELISA, this test remains very suitable for confirming positive cases (with high specificity), but a negative result (with limited sensitivity) requires further analysis before FMD can be ruled out.

**Antibody detection.** In the WRL, the liquid phase blocking ELISA was routinely used for FMD antibody detection until recently (Hamblin *et al.*, 1986a,b, 1987). The sensitivity of this assay is close to 100% and the specificity *c.* 95%. Samples giving inconclusive results are tested by a virus neutralization test (VNT) (Golding *et al.*, 1976). The relatively low specificity of the liquid phase ELISA makes the method less than optimal for large-scale screening purposes, as numerous confirmatory VNTs are likely to be required. Recently, a solid

phase competitive ELISA (SP-C ELISA) has been developed, validation tests showing high sensitivity and specificity (99.8%) at the chosen “cut-off” (Mackay *et al.*, 2001). This method detects all experimentally infected animals (cattle, sheep and pigs) at 5–8 days after infection and for several months thereafter.

As mentioned above, the VNT is used to investigate inconclusive antibody results obtained by ELISA. The specificity of the VNT at a dilution of 1 in 45 is 100% (Paiba *et al.*, submitted). At present the VNT is recommended by the OIE as the definitive “gold standard” for the final assessment of such results. It is possible that when sufficient validation data become available, some of the newer tests may replace the VNT.

In addition to these tests, pen-side tests for antibodies, including antibodies to non-structural proteins (see below), should be developed and validated for rapid on-site (*i.e.*, on-farm or near-farm) testing and to complement tests used for the detection of virus/antigen/genome.

It is important to note that although validation studies are in progress, neither the RT-PCR techniques nor the pen-side tests mentioned above have yet been approved by the OIE. This is essential for tests used to determine the FMD status of livestock or animal products (*e.g.*, ova or semen) moving in international trade.

#### *Distinguishing Infected from Vaccinated Stock*

At present there is no fully validated, OIE-approved test capable of making this distinction. Although probang sampling can identify carrier animals, it cannot be used to exclude the possibility of carriers because (a) the amounts of virus are low (often close to the limits of detection of cell culture assay systems) and decline over time, and (b) the excretion of virus by carriers is intermittent (Alexandersen *et al.*, 2002b). Moreover, no statistical sample frame has been established for probang sampling that can reliably demonstrate the absence of infection. Indeed, such a programme would probably be impracticable for large-scale surveillance, due to the amount of work required. In addition, the testing of antibodies to FMDV non-structural proteins (see below) gives no absolute guarantee of freedom, as a significant proportion of vaccinated carrier animals may fail to demonstrate an anti-NSP (non-structural protein) response (Mackay, 1998). However, this test could probably be used on a herd basis when it has been fully validated according to an established and statistically valid sampling frame.

*Tests to detect antibodies against the conserved, non-structural proteins (NSPs) of FMDV.* Such tests have been developed in several laboratories (Berger *et al.*, 1990; Neitzert *et al.*, 1991; Bergmann *et al.*, 1993; Lubroth and Brown, 1995; Mackay, 1998; Sorensen *et al.*, 1998; Shen *et al.*, 1999). These methods can be used to distinguish infected animals from vaccinated animals on a herd basis, but separate assays are required to test ruminants and pigs. Initial laboratory results are encouraging but, despite the recent successful development by some manufacturers of vaccines from which NSPs have been removed, more work is required to demonstrate that they satisfy validation criteria under field circumstances. Furthermore, in relation to carriers, the problem remains that some vaccinated carrier animals fail to develop antibodies against the NSPs, even though they are carrying live virus in their pharynx (Mackay, 1998). Thus, at present, tests for antibodies to NSPs cannot completely guarantee that a population of vaccinated animals exposed to live virus contains no carriers. In contrast, a non-vaccinated population can be screened by tests which detect antibodies to structural antigens and a negative result will exclude FMD in a statistically robust manner.

*Antibody and antibody isotype concentrations.* FMDV antibody titres cannot be used to distinguish between carriers and non-carriers, although carriers often have high titres (Salt and Ilott, 1996), because the variability is too great between the two populations. There may be a correlation between IgA concentration (in serum or OP fluid) and the carrier state (Salt *et al.*, 1996); however, this approach may not be reliable, since the administration of glucocorticoids (e.g., dexamethasone) has been shown to decrease the secretory IgA titres in carrier animals. Furthermore, dexamethasone treatment temporarily “removes” FMDV from the pharyngeal region of carriers, but when the treatment ceases the virus returns (Ilott *et al.*, 1997). The reasons for these phenomena are not known, but it has been speculated that the virus is associated with lymphoid cells which are depleted from the pharyngeal area during the steroid treatment. Based on our own studies of FMDV target cells *in vivo*, we consider it more likely that most of the virus in the pharynx is associated with epithelial cells, and that the dexamethasone treatment somehow changes the intracellular equilibrium to cause decreased production or release of virus. Dexamethasone and related compounds have a range of effects on cells (including epithelial cells), e.g., changes in the extent of cell division, the degree of differentiation and the production

levels of various cellular gene products (Boggaram *et al.*, 1989; Phelps and Floros, 1991; Young *et al.*, 1991; Boggaram and Margana, 1992; Veletza *et al.*, 1992; Levine *et al.*, 1993).

## Conclusions

FMD is an economically important, highly contagious disease of livestock. It can spread rapidly by a multitude of routes and affect both domesticated and wild ruminants and pigs. The disease is usually characterized by severe lameness and dullness in pigs and severe mouth lesions in cattle, but the signs may be mild, especially in sheep and goats. An important feature of FMD is persistent infection in ruminants, producing the so-called carrier state. This may occur in convalescent ruminant animals as well as in vaccinated ruminants following exposure to infectious virus. Although the amount of infectivity that can be recovered from carriers is small, the virus continues to be present in some species for months, and in others for years. The risk of transmission from carrier animals cannot be excluded, but it appears to be low and to require certain, as yet undefined, trigger factors. The severe and highly contagious nature of the disease and the recognition of a carrier state have had major adverse consequences for the international trade in livestock and animal products. New techniques show great potential for more rapid and effective diagnosis and surveillance, but much remains to be done to validate their performance before they can be adopted by regulatory authorities for routine use. Further research is urgently needed to increase understanding of the pathogenesis and epidemiology of FMD so that improved, scientifically sound strategies can be developed for disease control.

## Acknowledgments

We thank Melvyn Quan, Ciara Murphy, Raza Ahmed and Jeanette Knight for their contributions and Steven Archibald for artwork. The research was supported by the Department for Environment, Food and Rural Affairs (DEFRA) and The Biotechnology and Biological Sciences Research Council (BBSRC), UK.

## References

- Aggarwal, N., Zhang, Z., Cox, S., Statham, R., Alexandersen, S., Kitching, R. P. and Barnett, P. V. (2002). Experimental studies with foot-and-mouth disease

- virus, strain O, responsible for the 2001 epidemic in the United Kingdom. *Vaccine*, **20**, 2508–2515.
- Alexandersen, S., Brotherhood, I. and Donaldson, A. I. (2002a). Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O1 Lausanne. *Epidemiology and Infection*, **128**, 301–312.
- Alexandersen, S. and Donaldson, A. I. (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., Forsyth, M. A., Reid, S. M. and Belsham, G. J. (2000). Development of reverse transcription-PCR (oligonucleotide probing) enzyme-linked immunosorbent assays for diagnosis and preliminary typing of foot-and-mouth disease: a new system using simple and aqueous-phase hybridization. *Journal of Clinical Microbiology*, **38**, 4604–4613.
- Alexandersen, S., Kitching, R. P., Mansley, L. M. and Donaldson, A. I. (2003a). Clinical and laboratory investigations of five outbreaks during the early stages of the 2001 foot-and-mouth disease epidemic in the United Kingdom. *Veterinary Record*, **152**, 489–496.
- Alexandersen, S., Oleksiewicz, M. B. and Donaldson, A. I. (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., Quan, M., Murphy, C., Knight, J. and Zhang, Z. (2003b). 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*, in press.
- Alexandersen, S., Zhang, Z. and Donaldson, A. (2002b). Aspects of the persistence of foot-and-mouth disease virus in animals—the carrier problem. *Microbes and Infection*, **4**, 1099–1110.
- Alexandersen, S., Zhang, Z., Reid, S. M., Hutchings, G. H. and Donaldson, A. I. (2002c). 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.
- Amadori, M. and Melegari, M. (1987). Humoral and cellular parameters of experimental foot-and-mouth disease in heifers. *Zentralblatt für Veterinärmedizin [B]*, **34**, 480–488.
- Anderson, E. C., Doughty, W. J. and Anderson, J. (1976). The role of sheep and goats in the epizootiology of foot-and-mouth disease in Kenya. *Journal of Hygiene (London)*, **76**, 395–402.
- Anonymous (1969). *Report of the Committee of Inquiry on Foot-and-Mouth Disease* (1968), Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, Part 1.
- Anonymous (1986). *Foot-and-Mouth Disease Ageing of Lesions*, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, Reference Book 400.
- Bachrach, H. L. (1968). Foot-and-mouth disease. *Annual Review of Microbiology*, **22**, 201–244.
- Bachrach, H. L., Breese, S. S., Callis, J. J., Hess, W. R. and Patty, R. E. (1957). Inactivation of foot and mouth disease virus by pH and temperature changes and by formaldehyde. *Proceedings of the Society for Experimental Biology and Medicine*, **95**, 147–152.
- Badovinac, V. P., Porter, B. B. and Harty, J. T. (2002). Programmed contraction of CD8(+) T cells after infection. *Nature Immunology*, **3**, 619–626.
- Barnett, P. V. and Carabin, H. (2002). A review of emergency foot-and-mouth disease (FMD) vaccines. *Vaccine*, **20**, 1505–1514.
- Barnett, P. V. and Cox, S. J. (1999). The role of small ruminants in the epidemiology and transmission of foot-and-mouth disease. *Veterinary Journal*, **158**, 6–13.
- Bastos, A. D., Bertschinger, H. J., Cordel, C., van Vuuren, C. D., Keet, D., Bengis, R. G., Grobler, D. G. and Thomson, G. R. (1999). Possibility of sexual transmission of foot-and-mouth disease from African buffalo to cattle. *Veterinary Record*, **145**, 77–79.
- Bastos, A. D., Boshoff, C. I., Keet, D. F., Bengis, R. G. and Thomson, G. R. (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, P. W. (1995). Foot-and-mouth disease virus undergoes restricted replication in macrophage cell cultures following Fc receptor-mediated adsorption. *Virology*, **207**, 503–509.
- Beck, E. and Strohmaier, K. (1987). Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *Journal of Virology*, **61**, 1621–1629.
- Belsham, G. J. (1993). Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; aspects of virus protein synthesis, protein processing and structure. *Progress in Biophysics and Molecular Biology*, **60**, 241–260.
- Berger, H. G., Straub, O. C., Ahl, R., Tesar, M. and Marquardt, O. (1990). Identification of foot-and-mouth disease virus replication in vaccinated cattle by antibodies to non-structural virus proteins. *Vaccine*, **8**, 213–216.
- Bergmann, I. E., Malirat, V., Auge de Mello, V. and Gomes, I. (1996). Detection of foot-and-mouth disease viral sequences in various fluids and tissues during persistence of the virus in cattle. *American Journal of Veterinary Research*, **57**, 134–137.
- Bergmann, I. E., de Mello, P. A., Neitzert, E., Beck, E. and Gomes, I. (1993). Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransfer blot analysis with bioengineered non-structural viral antigens. *American Journal of Veterinary Research*, **54**, 825–831.
- Black, L., Francis, M. J., Rweyemamu, M. M., Umehara, O. and Boge, A. (1984). The relationship between

- serum antibody titres and protection from foot and mouth disease in pigs after oil emulsion vaccination. *Journal of Biological Standardization*, **12**, 379–389.
- Boggaram, V. and Margana, R. K. (1992). Rabbit surfactant protein C: cDNA cloning and regulation of alternatively spliced surfactant protein C mRNAs. *American Journal of Physiology and Lung Cellular and Molecular Physiology*, **263**, L634–L644.
- Boggaram, V., Smith, M. E. and Mendelson, C. R. (1989). Regulation of expression of the gene encoding the major surfactant protein (SP-A) in human fetal lung in vitro. Disparate effects of glucocorticoids on transcription and on mRNA stability. *Journal of Biological Chemistry*, **264**, 11421–11427.
- Borgen, H. C. and Schwobel, W. (1964). A plaque marker of type C foot-and-mouth disease virus strains from a porcine epizootic. *Nature (London)*, **202**, 932–933.
- Brown, F. and Cartwright, B. (1960). Purification of the virus of foot-and-mouth disease by fluorocarbon treatment and its dissociation from neutralizing antibody. *Journal of Immunology*, **85**, 309–313.
- Brown, C. C., Chinsangaram, J. and Grubman, M. J. (2000). Type I interferon production in cattle infected with 2 strains of foot-and-mouth disease virus, as determined by in situ hybridization. *Canadian Journal of Veterinary Research*, **64**, 130–133.
- Brown, C. C., Meyer, R. F., Olander, H. J., House, C. and Mebus, C. A. (1992). A pathogenesis study of foot-and-mouth disease in cattle, using in situ hybridization. *Canadian Journal of Veterinary Research*, **56**, 189–193.
- Brown, C. C., Olander, H. J. and Meyer, R. F. (1995). Pathogenesis of foot-and-mouth disease in swine, studied by in-situ hybridization. *Journal of Comparative Pathology*, **113**, 51–58.
- Brown, C. C., Piccone, M. E., Mason, P. W., McKenna, T. S. and Grubman, M. J. (1996). Pathogenesis of wild-type and leaderless foot-and-mouth disease virus in cattle. *Journal of Virology*, **70**, 5638–5641.
- Burrows, R. (1966). Studies on the carrier state of cattle exposed to foot-and-mouth disease virus. *Journal of Hygiene (London)*, **64**, 81–90.
- Burrows, R. (1966). The infectivity assay of foot-and-mouth disease virus in pigs. *Journal of Hygiene (London)*, **64**, 419–429.
- Burrows, R. (1968). Excretion of foot-and-mouth disease virus prior to the development of lesions. *Veterinary Record*, **83**, 387–388.
- Burrows, R. (1968). The persistence of foot-and-mouth disease virus in sheep. *Journal of Hygiene (London)*, **66**, 633–640.
- Burrows, R., Mann, J. A., Garland, A. J., 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.
- Burrows, R., Mann, J. A., Greig, A., Chapman, W. G. and Goodridge, D. (1971). The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *Journal of Hygiene (London)*, **69**, 307–321.
- Callahan, J. D., Brown, F., Osorio, F. A., Sur, J. H., Kramer, E., Long, G. W., Lubroth, J., Ellis, S. J., Shoulars, K. S., Gaffney, K. L., Rock, D. L. and Nelson, W. M. (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 Medicine Association*, **220**, 1636–1642.
- Callens, M. and De Clercq, K. (1997). Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. *Journal of Virological Methods*, **67**, 35–44.
- 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.
- Childerstone, A. J., Cedillo-Baron, L., Foster-Cuevas, M. and Parkhouse, R. M. (1999). Demonstration of bovine CD8 + T-cell responses to foot-and-mouth disease virus. *Journal of General Virology*, **80**, 663–669.
- Chinsangaram, J., Piccone, M. E. and Grubman, M. J. (1999). Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *Journal of Virology*, **73**, 9891–9898.
- Coetzer, J. A. W., Thomsen, G. R., Tustin, R. C. and Kriek, N. P. J. (1994). Foot-and-mouth disease. In: *Infectious Diseases of Livestock with Special Reference to Southern Africa*, J. A. W., Coetzer, G. R., Thomsen, R. C., Tustin and N. P. J., Kriek (Eds) Oxford University Press, Cape Town, pp. 825–852.
- Condy, J. B., Hedger, R. S., Hamblin, C. and Barnett, I. T. (1985). The duration of the foot-and-mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd. *Comparative Immunology and Microbiology of Infectious Diseases*, **8**, 259–265.
- Cottral, G. E. (1969). Persistence of foot-and-mouth disease virus in animals, their products and the environment. *Bulletin de l'Office International des Epizooties*, **70**, 549–568.
- Cottral, G. E. and Bachrach, H. L. (1968). Foot-and-mouth disease viremia. *Proceedings of the Annual Meeting of the United States Animal Health Association*, **72**, 383–399.
- Cottral, G. E., Gailiunas, P. and Campion, R. L. (1963). Detection of foot-and-mouth disease virus in lymph nodes of cattle throughout course of infection. *Proceedings of the Annual Meeting of the United States Livestock Sanitary Association*, **67**, 463–472.
- Cottral, G. E., Gailiunas, P. and Cox, B. F. (1968). Foot-and-mouth disease virus in semen of bulls and its transmission by artificial insemination. *Archiv für die Gesamte Virusforschung*, **23**, 362–377.
- Cottral, G. E., Patty, R. E., Gailiunas, P. and Scott, F. W. (1965). Sensitivity of cell cultures, cattle, mice, and guinea-pigs for detection of nineteen foot-and-mouth



- disease viruses. *Bulletin de l'Office International des Épidémiologies*, **63**, 1607–1625.
- Cottral, G. E., Patty, R. E., Gailunas, P. and Scott, F. W. (1966). Relationship of foot-and-mouth disease virus plaque size on cell cultures to infectivity for cattle by intramuscular inoculation. *Archiv für die Gesamte Virusforschung*, **18**, 276–293.
- Dawe, P. S., Flanagan, F. O., Madekurozwa, R. L., Sorensen, K. J., Anderson, E. C., Foggin, C. M., Ferris, N. P. and Knowles, N. J. (1994a). Natural transmission of foot-and-mouth disease virus from African buffalo (*Syncerus caffer*) to cattle in a wildlife area of Zimbabwe. *Veterinary Record*, **134**, 230–232.
- Dawe, P. S., Sorensen, K., Ferris, N. P., Barnett, I. T., Armstrong, R. M. and Knowles, N. J. (1994b). Experimental transmission of foot-and-mouth disease virus from carrier African buffalo (*Syncerus caffer*) to cattle in Zimbabwe. *Veterinary Record*, **134**, 211–215.
- De Castro, M. P. (1964). Behaviour of the foot and mouth disease virus in cell cultures: susceptibility of the IB-RS-2 cell line. *Arquivos do Instituto Biológico (Sao Paulo)*, **31**, 63–78.
- De Castro, M. P. and Pisani, R. C. B. (1964). The chromosome complement of the IB-RS-2 swine cell line susceptible to the foot and mouth disease virus. *Arquivos do Instituto Biológico (Sao Paulo)*, **31**, 155–166.
- Donaldson, A. I. (1979). Airborne foot-and-mouth disease. *Veterinary Bulletin*, **49**, 653–659.
- Donaldson, A. I. (1986). Aerobiology of foot-and-mouth disease (FMD): an outline and recent advances. *Revue Scientifique et Technique de l'Office International des Épidémiologies*, **5**, 315–321.
- Donaldson, A. I. (1987). Foot-and-mouth disease: the principal features. *Irish Veterinary Journal*, **41**, 325–327.
- Donaldson, A. I. (1997). Risks of spreading foot and mouth disease through milk and dairy products. *Revue Scientifique et Technique de l'Office International des Épidémiologies*, **16**, 117–124.
- Donaldson, A. I. (1998). Experimental and natural adaptation of strains of foot-and-mouth disease virus to different species. *Session of the Research Group of the Standing Technical Committee, European Commission for the Control of Foot-and-Mouth Disease*, pp. 18–22.
- Donaldson, A. I. and Alexandersen, S. (2001). The relative resistance of pigs to infection by natural aerosols of foot-and-mouth disease virus. *Veterinary Record*, **148**, 600–602.
- Donaldson, A. I. and Alexandersen, S. (2002). Predicting the spread of foot and mouth disease by airborne virus. *Revue Scientifique et Technique de l'Office International des Épidémiologies*, **21**, 569–575.
- Donaldson, A. I. and Alexandersen, S. (2003). The virological determinants of the epidemiology of foot-and-mouth disease. In: *Foot-and-Mouth Diseases: Control Strategies*. B. Dodet (Ed.) Éditions scientifiques et médicales Elsevier SAS (the Mérieux Foundation), in press.
- Donaldson, A. I., Alexandersen, S., Sorensen, J. H. and Mikkelsen, T. (2001). The relative risks of the uncontrollable (airborne) spread of foot-and-mouth disease by different species. *Veterinary Record*, **148**, 602–604.
- Donaldson, A. I. and Ferris, N. P. (1980). Sites of release of airborne foot-and-mouth disease virus from infected pigs. *Research in Veterinary Science*, **29**, 315–319.
- Donaldson, A. I., Ferris, N. P. and Gloster, J. (1982a). Air sampling of pigs infected with foot-and-mouth disease virus: comparison of Litton and cyclone samplers. *Research in Veterinary Science*, **33**, 384–385.
- Donaldson, A. I., Ferris, N. P. and Wells, G. A. (1984). Experimental foot-and-mouth disease in fattening pigs, sows and piglets in relation to outbreaks in the field. *Veterinary Record*, **115**, 509–512.
- Donaldson, A. I., Gibson, C. F., Oliver, R., Hamblin, C. and Kitching, R. P. (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.
- Donaldson, A. I., Gloster, J., Harvey, L. D. and Deans, D. H. (1982b). Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. *Veterinary Record*, **110**, 53–57.
- Donaldson, A. I., Herniman, K. A., Parker, J. and Sellers, R. F. (1970). Further investigations on the airborne excretion of foot-and-mouth disease virus. *Journal of Hygiene (London)*, **68**, 557–564.
- Donaldson, A. I., Kitching, P. and Barnett, I. T. (1996). Foot-and-mouth disease. In: *Manual of Standards for Diagnostic Tests and Vaccines*, OIE, Paris, pp. 47–56.
- Donaldson, A. I. and Sellers, R. F. (2000). Foot-and-mouth disease. In: *Diseases of Sheep*, 3rd Edn, W. B. Martin and I. D. Aitken (Eds) Blackwell Science, Oxford, pp. 254–258.
- Donn, A. (1993). Pathogenesis of persistence of foot-and-mouth disease virus in experimentally infected cattle and in a model cell system. PhD Thesis, University of Hertfordshire.
- Donn, A., Castagnaro, M. and Donaldson, A. I. (1995). Ultrastructural and replicative features of foot-and-mouth disease virus in persistently infected BHK-21 cells. *Archives of Virology*, **140**, 13–25.
- Donn, A., Martin, L. A. and Donaldson, A. I. (1994). Improved detection of persistent foot-and-mouth disease infection in cattle by the polymerase chain reaction. *Journal of Virological Methods*, **49**, 179–186.
- Dunn, C. S. and Donaldson, A. I. (1997). Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. *Veterinary Record*, **141**, 174–175.
- Eskildsen, M. (1969). Experimental pulmonary infection of cattle with foot-and-mouth disease virus. *Nordisk Veterinær Medicin*, **21**, 86–91.
- Fellowes, O. N. and Suttmoller, P. (1970). Foot-and-mouth disease virus: biological characteristics of virus from bovine carriers. *Archiv für die Gesamte Virusforschung*, **30**, 173–180.
- Ferguson, N. M., Donnelly, C. A. and Anderson, R. M. (2001). The foot-and-mouth epidemic in Great

- Britain: pattern of spread and impact of interventions. *Science*, **292**, 1155–1160.
- Ferris, N. P. 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, N. P., Kitching, R. P., Oxtoby, J. M., Philpot, R. M. and Rendle, R. (1990). Use of inactivated foot-and-mouth disease virus antigen in liquid-phase blocking ELISA. *Journal of Virological Methods*, **29**, 33–41.
- Ferris, N. P., Powell, H. and Donaldson, A. I. (1988). Use of pre-coated immunoPlate and freeze-dried reagents for the diagnosis of foot-and-mouth disease and swine vesicular disease by enzyme-linked immunosorbent assay (ELISA). *Journal of Virological Methods*, **19**, 197–206.
- Francis, M. J. and Black, L. (1983). Antibody response in pig nasal fluid and serum following foot-and-mouth disease infection or vaccination. *Journal of Hygiene (London)*, **91**, 329–334.
- Francis, M. J., Ouldrige, E. J. and Black, L. (1983). Antibody response in bovine pharyngeal fluid following foot-and-mouth disease vaccination and, or, exposure to live virus. *Research in Veterinary Science*, **35**, 206–210.
- Fry, E. E., Lea, S. M., Jackson, T., Newman, J. W., Ellard, F. M., Blakemore, W. E., Abu-Ghazaleh, R., Samuel, A., King, A. M. and Stuart, D. I. (1999). The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. *EMBO Journal*, **18**, 543–554.
- Gailiunas, P. (1968). Microscopic skin lesions in cattle with foot-and-mouth disease. *Archiv für die Gesamte Virusforschung*, **25**, 188–200.
- Garland, A. J., (1974). The inhibitory activity of secretions in cattle against FMDV. PhD Thesis, University of London.
- Garland, A. J. M. and Donaldson, A. I. (1990). Foot-and-mouth disease. *Surveillance*, **17**, 6–8.
- Gibbs, E. P., Herniman, K. A., Lawman, M. J. and Sellers, R. F. (1975). Foot-and-mouth disease in British deer: transmission of virus to cattle, sheep and deer. *Veterinary Record*, **96**, 558–563.
- Gibson, C. F. and Donaldson, A. I. (1986). Exposure of sheep to natural aerosols of foot-and-mouth disease virus. *Research in Veterinary Science*, **41**, 45–49.
- Gloster, J., Blackall, J., Sellers, R. F. and Donaldson, A. I. (1981). Forecasting the spread of foot-and-mouth disease. *Veterinary Record*, **108**, 370–374.
- Gloster, J., Sellers, R. F. and Donaldson, A. I. (1982). Long distance transport of foot-and-mouth disease virus over the sea. *Veterinary Record*, **110**, 47–52.
- Golding, S. M., Hedger, R. S., Talbot, P. and Watson, J. (1976). Radial immuno-diffusion and serum-neutralization techniques for the assay of antibodies to SVD. *Research in Veterinary Science*, **20**, 142–147.
- Gurhan, S. I., Gurhan, B., Osturkmen, A., Aynagoz, G., Candas, A. and Kizil, S. (1993). Establishment of the prevalence of persistently infected cattle and sheep in Anatolia with FMDV. *Etlik Veteriner Mikrobiyologii Dergisi*, **7**, 52–59.
- Hamblin, C., Armstrong, R. M. and Hedger, R. S. (1984). A rapid enzyme-linked immunosorbent assay for the detection of foot-and-mouth disease virus in epithelial tissues. *Veterinary Microbiology*, **9**, 435–443.
- Hamblin, C., Barnett, I. T. and Crowther, J. R. (1986a). A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. II. Application. *Journal of Immunological Methods*, **93**, 123–129.
- Hamblin, C., Barnett, I. T. and Hedger, R. S. (1986b). A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method of ELISA. *Journal of Immunological Methods*, **93**, 115–121.
- Hamblin, C., Kitching, R. P., Donaldson, A. I., Crowther, J. R. and Barnett, I. T. (1987). Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. III. Evaluation of antibodies after infection and vaccination. *Epidemiology and Infection*, **99**, 733–744.
- Harper, G. J., (1968). Tests made to measure the number of microorganisms escaping the vacuum pump outlet of a bulk collection milk tanker. Ministry of Defence, Microbiological Research Establishment, Porton Down, UK, p. 15.
- Harty, J. T. and Badovinac, V. P. (2002). Influence of effector molecules on the CD8(+) T cell response to infection. *Current Opinion in Immunology*, **14**, 360–365.
- Hatch, T. F. and Gross, P. (1964). *Pulmonary Deposition and Retention of Inhaled Aerosols*, Academic Press, London, pp. 45–68.
- Hearps, A., Zhang, Z. and Alexandersen, S. (2002). Evaluation of the portable Cepheid SmartCycler real-time PCR machine for the rapid diagnosis of foot-and-mouth disease. *Veterinary Record*, **150**, 625–628.
- Hedger, R. S. (1968). The isolation and characterization of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. *Journal of Hygiene (London)*, **66**, 27–36.
- Hedger, R. S. (1970). Observations on the carrier state and related antibody titres during an outbreak of foot-and-mouth disease. *Journal of Hygiene (London)*, **68**, 53–60.
- Hedger, R. S. (1971). The carrier state in foot-and-mouth disease and the probang test. *State Veterinary Journal*, **26**, 45–50.
- Hedger, R. S. (1972). Foot-and-mouth disease and the African buffalo (*Syncerus caffer*). *Journal of Comparative Pathology*, **82**, 19–28.
- Hedger, R. S. and Condry, J. B. (1985). Transmission of foot-and-mouth disease from African buffalo virus carriers to bovines. *Veterinary Record*, **117**, 205.
- Hedger, R. S., Condry, J. B. and Golding, S. M. (1972). Infection of some species of African wild life with foot-and-mouth disease virus. *Journal of Comparative Pathology*, **82**, 455–461.

- Hedger, R. S. and Dawson, P. S. (1970). Foot-and-mouth disease virus in milk: an epidemiological study. *Veterinary Record*, **87**, 186–188.
- Hedger, R. S. and Stubbins, A. G. J. (1971). The carrier state in FMD and the probang test. *State Veterinary Journal*, **26**, 45–50.
- Henderson, W. M. (1948). Further consideration of some of the factors concerned in intracutaneous injection of cattle. *Journal of Pathology and Bacteriology*, **60**, 137–139.
- Henderson, W. M. (1949). *The Quantitative Study of Foot-and-Mouth Disease Virus*, Her Majesty's Stationery Office, London, p. 8.
- Henderson, W. M. (1952). A comparison of different routes of inoculation of cattle for detection of the virus of foot-and-mouth disease. *Journal of Hygiene (London)*, **50**, 182–194.
- Hess, W. R., Bachrach, H. L. and Callis, J. J. (1967). Persistence of foot-and-mouth disease virus in bovine kidneys and blood as related to the occurrence of antibodies. *American Journal of Veterinary Research*, **21**, 1104–1108.
- Hofner, M. C., Fosbery, M. W., Eckersall, P. D. and Donaldson, A. I. (1994). Haptoglobin response of cattle infected with foot-and-mouth disease virus. *Research in Veterinary Science*, **57**, 125–128.
- House, C. and Meyer, R. F. (1993). The detection of foot-and-mouth disease virus in oesophageal-pharyngeal samples by a polymerase chain reaction technique. *Journal of Virological Methods*, **43**, 1–6.
- Hughes, G. J., Mioulet, V., Kitching, R. P., Woolhouse, M. E., Alexandersen, S. and Donaldson, A. I. (2002). Foot-and-mouth disease virus infection of sheep: implications for diagnosis and control. *Veterinary Record*, **150**, 724–727.
- Hyslop, N. S. (1965). Secretion of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle. *Journal of Comparative Pathology*, **75**, 111–117.
- Hyslop, N. S. (1970). The epizootiology and epidemiology of foot and mouth disease. *Advances in Veterinary Science and Comparative Medicine*, **14**, 261–307.
- Ilott, M. C., Salt, J. S., Gaskell, R. M. and Kitching, R. P. (1997). Dexamethasone inhibits virus production and the secretory IgA response in oesophageal-pharyngeal fluid in cattle persistently infected with foot-and-mouth disease virus. *Epidemiology and Infection*, **118**, 181–187.
- Jackson, T., Blakemore, W., Newman, J. W., Knowles, N. J., Mould, A. P., Humphries, M. J. and King, A. M. (2000a). Foot-and-mouth disease virus is a ligand for the high-affinity binding conformation of integrin  $\alpha 5 \beta 1$ : influence of the leucine residue within the RGD motif on selectivity of integrin binding. *Journal of General Virology*, **81** (Pt. 5), 1383–1391.
- Jackson, T., Mould, A. P., Sheppard, D. and King, A. M. (2002). Integrin  $\alpha \text{HV} \beta 1$  is a receptor for foot-and-mouth disease virus. *Journal of Virology*, **76**, 935–941.
- Jackson, T., Sharma, A., Ghazaleh, R. A., Blakemore, W. E., Ellard, F. M., Simmons, D. L., Newman, J. W., Stuart, D. I. and King, A. M. (1997). Arginine-glycine-aspartic acid-specific binding by foot-and-mouth disease viruses to the purified integrin  $\alpha (v) \beta 3$  in vitro. *Journal of Virology*, **71**, 8357–8361.
- Jackson, T., Sheppard, D., Denyer, M., Blakemore, W. and King, A. M. (2000b). The epithelial integrin  $\alpha \text{HV} \beta 6$  is a receptor for foot-and-mouth disease virus. *Journal of Virology*, **74**, 4949–4956.
- Jones, A. L. (1965). Growth of foot and mouth disease virus in organ cultures of mouse pancreas. *Nature*, **207**, 665–666.
- Kaaden, O., Eissner, G. and Bohm, H. O. (1973). Isolierung eines MKS-dauerausscheidervirus typ A aus einem rind. *Deutsche Tierärztliche Wochenschrift*, **80**, 269–271.
- Kaaden, O., Eissner, G. and Bohm, H. O. (1975). Studies on permanent virus excretors in cattle vaccinated and experimentally infected with foot-and-mouth disease. *Animal Research Development*, **1**, 20–33.
- Kitching, R. P. (1998). A recent history of foot-and-mouth disease. *Journal of Comparative Pathology*, **118**, 89–108.
- Kitching, R. P. and Alexandersen, S. (2002). Clinical variation in foot and mouth disease: pigs. *Revue Scientifique et Technique de l'Office International des Epizooties*, **21**, 513–518.
- Kitching, R. P., Knowles, N. J., Samuel, A. R. and Donaldson, A. I. (1989). Development of foot-and-mouth disease virus strain characterisation—a review. *Tropical Animal Health and Production*, **21**, 153–166.
- Knowles, N. J., Davies, P. R., Henry, T., O'Donnell, V., Pacheco, J. M. and Mason, P. W. (2001a). Emergence in Asia of foot-and-mouth disease viruses with altered host range: characterization of alterations in the 3A protein. *Journal of Virology*, **75**, 1551–1556.
- Knowles, N. J., Samuel, A. R., Davies, P. R., Kitching, R. P. and Donaldson, A. I. (2001b). Outbreak of foot-and-mouth disease virus serotype O in the UK caused by a pandemic strain. *Veterinary Record*, **148**, 258–259.
- Korn, G. (1957). Experimental studies of the demonstration of virus during the incubation period of foot-and-mouth disease and of its pathogenesis. *Archives of Experimental Veterinary Medicine*, **11**, 637–649.
- de Leeuw, P. W., Van Bakkum, J. G. and Tiessink, J. W. (1978). Excretion of foot-and-mouth disease virus in oesophageal-pharyngeal fluid and milk of cattle after intranasal infection. *Journal of Hygiene (London)*, **81**, 415–425.
- Levine, S. J., Larivée, P., Logun, C., Angus, C. W. and Shelhamer, J. H. (1993). Corticosteroids differentially regulate secretion of IL-6, IL-8, and G-CSF by a human bronchial epithelial cell line. *American Journal*

- of Physiology and Lung Cellular and Molecular Physiology*, **265**, L360–L368.
- Locher, F., Suryanarayana, V. V. and Tratschin, J. D. (1995). Rapid detection and characterization of foot-and-mouth disease virus by restriction enzyme and nucleotide sequence analysis of PCR products. *Journal of Clinical Microbiology*, **33**, 440–444.
- Lubroth, J. and Brown, F. (1995). Identification of native foot-and-mouth disease virus non-structural protein 2C as a serological indicator to differentiate infected from vaccinated livestock. *Research in Veterinary Science*, **59**, 70–78.
- Mackay, D. K. (1998). Differentiating infection from vaccination in foot-and-mouth disease. *Veterinary Quarterly*, **20** (Suppl. 2), 2–5.
- Mackay, D. K., Bulut, A. N., Rendle, T., Davidson, F. and Ferris, N. P. (2001). A solid-phase competition ELISA for measuring antibody to foot-and-mouth disease virus. *Journal of Virological Methods*, **97**, 33–48.
- Manuel-Leon, A. and Casal, J. (2001). Application of a probabilistic approach to the risk assessment of virus airborne transmission. *Veterinary Record*, **148**, 574–575.
- Maragon, S., Facchin, E., Moutou, F., Massirio, I., Vincenzi, G. and Davies, G. (1994). The 1993 Italian foot-and-mouth disease epidemic: epidemiological features of the four outbreaks identified in Verona province (Veneto region). *Veterinary Record*, **135**, 53–57.
- McColl, K. A., Westbury, H. A., Kitching, R. P. and Lewis, V. M. (1995). The persistence of foot-and-mouth disease virus on wool. *Australian Veterinary Journal*, **72**, 286–292.
- McCullough, K. C., De Simone, F., Brocchi, E., Capucci, L., Crowther, J. R. and Kihm, U. (1992). Protective immune response against foot-and-mouth disease. *Journal of Virology*, **66**, 1835–1840.
- McKenna, T. S., Lubroth, J., Rieder, E., Baxt, B. and Mason, P. W. (1995). Receptor binding site-deleted foot-and-mouth disease (FMD) virus protects cattle from FMD. *Journal of Virology*, **69**, 5787–5790.
- McVicar, J. W., Graves, J. H. and Suttmoller, P. (1970). Growth of foot-and-mouth disease virus in the bovine pharynx. *Proceedings of the Annual Meeting of the United States Animal Health Association*, **74**, 230–234.
- McVicar, J. W., McKercher, P. D., and Graves, J. H., (1976). The influence of infectious bovine rhinotracheitis virus on the foot-and-mouth disease carrier state. *Proceedings of the Annual Meeting of the United States Animal Health Association*, **80**, pp. 254–261.
- McVicar, J. W., Richmond, J. Y., Campbell, C. H. and Hamilton, L. D. (1973). Observations of cattle, goats and pigs after administration of synthetic interferon inducers and subsequent exposure to foot and mouth disease virus. *Canadian Journal of Comparative Medicine*, **37**, 362–368.
- McVicar, J. W. and Suttmoller, P. (1969a). Sheep and goats as foot-and-mouth disease carriers. *Proceedings of the Annual Meeting of the United States Livestock Sanitary Association*, **73**, pp. 400–406.
- McVicar, J. W. and Suttmoller, P. (1969b). The epizootiological importance of foot-and-mouth disease carriers. II. The carrier status of cattle exposed to foot-and-mouth disease following vaccination with an oil adjuvant inactivated virus vaccine. *Archiv für die Gesamte Virusforschung*, **26**, 217–224.
- McVicar, J. W. and Suttmoller, P. (1971). Foot-and-mouth disease in sheep and goats: early virus growth in the pharynx and udder. *Proceedings of the Annual Meeting of the United States Animal Health Association*, **75**, 194–199.
- McVicar, J. W. and Suttmoller, P. (1974). Neutralizing activity in the serum and oesophageal-pharyngeal fluid of cattle after exposure to foot-and-mouth disease virus and subsequent re-exposure. *Archiv für die Gesamte Virusforschung*, **44**, 173–176.
- McVicar, J. W. and Suttmoller, P. (1976). Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation. *Journal of Hygiene (London)*, **76**, 467–481.
- McVicar, J.W., Suttmoller, P., Ferris, D.H. and Campbell, C.H., (1974). Foot-and-mouth disease in white-tailed deer: clinical signs and transmission in the laboratory. *Proceedings of the Annual Meeting of the United States Animal Health Association*, **78**, pp. 169–180.
- Mezencio, J. M., Babcock, G. D., Kramer, E. and Brown, F. (1999). Evidence for the persistence of foot-and-mouth disease virus in pigs. *Veterinary Journal*, **157**, 213–217.
- Mohanty, G. C. and Cottral, G. E. (1971). Foot-and-mouth disease virus carrier state in cells cultured from tissues of convalescent cattle. *Archiv für die Gesamte Virusforschung*, **34**, 1–13.
- 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.
- Moussa, A. A., Daoud, A., Tawfik, S., Omar, A., Azab, A. and Hassan, N. A. (1979). Susceptibility of water-buffaloes to infection with foot-and-mouth disease virus. *Journal of the Egyptian Veterinary Medical Association*, **39**, 65–83.
- Murphy, M. L., Forsyth, M. A., Belsham, G. J. and Salt, J. S. (1999). Localization of foot-and-mouth disease virus RNA by in situ hybridization within bovine tissues. *Virus Research*, **62**, 67–76.
- Neff, S., Mason, P. W. and Baxt, B. (2000). High-efficiency utilization of the bovine integrin  $\alpha(v)\beta(3)$  as a receptor for foot-and-mouth disease virus is dependent on the bovine  $\beta(3)$  subunit. *Journal of Virology*, **74**, 7298–7306.
- Neff, S., Sa-Carvalho, D., Rieder, E., Mason, P. W., Blystone, S. D., Brown, E. J. and Baxt, B. (1998). Foot-and-mouth disease virus virulent for cattle utilizes the integrin  $\alpha(v)\beta(3)$  as its receptor. *Journal of Virology*, **72**, 3587–3594.



- Neitzert, E., Beck, E., de Mello, P. A., Gomes, I. and Bergmann, I. E. (1991). Expression of the aphtho-virus RNA polymerase gene in *Escherichia coli* and its use together with other bioengineered nonstructural antigens in detection of late persistent infections. *Virology*, **184**, 799–804.
- Oleksiewicz, M. B., Donaldson, A. I. 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, R. E., Donaldson, A. I., Gibson, C. F., Roeder, P. L., Blanc Smith, P. M. 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.
- Parker, J. (1971). Presence and inactivation of foot-and-mouth disease virus in animal faeces. *Veterinary Record*, **88**, 659–662.
- Phelps, D. S. and Floros, J. (1991). Dexamethasone in vivo raises surfactant protein B mRNA in alveolar and bronchiolar epithelium. *American Journal of Physiology and Lung Cellular and Molecular Physiology*, **260**, L146–L152.
- Platt, H. (1961). Phagocytic activity in squamous epithelia and its role in cellular susceptibility to foot-and-mouth disease. *Nature*, **190**, 1075–1076.
- Prato Murphy, M. L., Meyer, R. F., Mebus, C., Schudel, A. A. and Rodriguez, M. (1994). Analysis of sites of foot and mouth disease virus persistence in carrier cattle via the polymerase chain reaction. *Archives of Virology*, **136**, 299–307.
- Reece, J. F. and Chawla, S. K. (2001). Prolapse of the soft palate in a male Arabian camel (*Camelus dromedarius*). *Veterinary Record*, **149**, 656–657.
- Reid, S. M., Ferris, N. P., Bruning, A., Hutchings, G. H., Kowalska, Z. and Akerblom, L. (2001a). 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, S., Ferris, N., Hutchings, G., Zhang, Z., Belsham, G. 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, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z., Belsham, G. J. and Alexandersen, S. (2001b). Diagnosis of foot-and-mouth disease by real-time fluorogenic PCR assay. *Veterinary Record*, **149**, 621–623.
- Reid, S. M., Forsyth, M. A., Hutchings, G. H. and Ferris, N. P. (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, S. M., Grierson, S. S., Ferris, N. P., Hutchings, G. H. and Alexandersen, S. (2003). Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *Journal of Virological Methods*, **107**, 129–139.
- Reid, S. M., Hutchings, G. H., Ferris, N. P. 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.
- Richmond, J. Y. (1971). Mouse resistance against foot-and-mouth disease virus induced by injections of Pyran. *Infection and Immunity*, **3**, 249–253.
- Rieder, E., Baxt, B. and Mason, P. W. (1994). Animal-derived antigenic variants of foot-and-mouth disease virus type A12 have low affinity for cells in culture. *Journal of Virology*, **68**, 5296–5299.
- Rieder, E., Berinstein, A., Baxt, B., Kang, A. and Mason, P. W. (1996). Propagation of an attenuated virus by design: engineering a novel receptor for a noninfectious foot-and-mouth disease virus. *Proceedings of the National Academy of Sciences of the USA*, **93**, 10428–10433.
- Rigden, R. C., Carrasco, C. P. and McCullough, K. C. (2000). Macrophages can act as infectious carriers of non-monocytotrophic viruses. *Immunology*, **101** (Suppl. 1), 124.
- Rigden, R. C., Carrasco, C. P., Summerfield, A. and McCullough, K. C. (2002). Macrophage phagocytosis of foot-and-mouth disease virus may create infectious carriers. *Immunology*, **106**, 537–548.
- Rodriguez, A., Martinez-Salas, E., Dopazo, J., Davila, M., Saiz, J. C. and Sobrino, F. (1992). Primer design for specific diagnosis by PCR of highly variable RNA viruses: typing of foot-and-mouth disease virus. *Virology*, **189**, 363–367.
- Roeder, P. L. and Le Blanc Smith, P. M. (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.
- Sa-Carvalho, D., Rieder, E., Baxt, B., Rodarte, R., Tanuri, A. and Mason, P. W. (1997). Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. *Journal of Virology*, **71**, 5115–5123.
- Salt, J. S. (1993). The carrier state in foot and mouth disease—an immunological review. *British Veterinary Journal*, **149**, 207–223.
- Salt, J. S. (1998). Persistent infection with foot-and-mouth disease virus. *Topics in Tropical Virology*, **1**, 77–128.
- Salt, J. S. and Illott, M. C. (1996). Virus titres in the development of FMDV persistence in cattle. *Session of the Research Group of the Standing Technical Committee, European Commission for the Control of Foot-and-Mouth Disease*, pp. 45–51.
- Salt, J. S., Mulcahy, G. and Kitching, R. P. (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.
- Sanson, R. L. (1994). The epidemiology of foot-and-mouth disease: implications for New Zealand. *New Zealand Veterinary Journal*, **42**, 41–53.
- Sanz-Parra, A., Sobrino, F. and Ley, V. (1998). Infection with foot-and-mouth disease virus results in a rapid reduction of MHC class I surface expression. *Journal of General Virology*, **79**, 433–436.
- Scott, F. W., Cottral, G. E. and Gailiunas, P. (1965). Presence of foot-and-mouth disease virus in the pituitary and central nervous system of experimentally infected cattle. *Proceedings of the Annual Meeting of the United States Livestock Sanitary Association*, **69**, 87–93.
- Scott, F. W., Cottral, G. E. and Gailiunas, P. (1966). Persistence of foot-and-mouth disease virus in external lesions and saliva of experimentally infected cattle. *American Journal of Veterinary Research*, **27**, 1531–1536.
- Seibold, H. R., Cottral, G. E., Patty, R. E. and Gailiunas, P. (1964). Apparent modification of foot-and-mouth disease virus after prolonged residence in surviving cells. *American Journal of Veterinary Research*, **25**, 806–814.
- Sellers, R. F. (1963). Multiplication, interferon production and sensitivity of virulent and attenuated strains of the virus of foot-and-mouth disease. *Nature*, **198**, 1228–1229.
- Sellers, R. F. (1971). Quantitative aspects of the spread of foot and mouth disease. *Veterinary Bulletin*, **41**, 431–439.
- Sellers, R. F., Burrows, R., Garland, A. J., Greig, A. and Parker, J. (1969). Exposure of vaccinated bulls and steers to airborne infection with foot-and-mouth disease. *Veterinary Record*, **85**, 198–199.
- Sellers, R. F., Burrows, R., Mann, J. A. and Dawe, P. (1968). Recovery of virus from bulls affected with foot-and-mouth disease. *Veterinary Record*, **83**, 303.
- Sellers, R. F., Burt, L. M., Cumming, A. and Stewart, D. L. (1959). The behaviour of strains of the virus of foot-and-mouth disease in pig, calf, ox and lamb kidney tissue cultures. *Archiv für die Gesamte Virusforschung*, **9**, 637–646.
- Sellers, R. F. and Forman, A. J. (1973). The Hampshire epidemic of foot-and-mouth disease, 1967. *Journal of Hygiene (London)*, **71**, 15–34.
- Sellers, R. F., Herniman, K. A. and Hawkins, C. W. (1972). The effect of a synthetic anionic polymer (pyran) on the development of foot-and-mouth disease in guinea-pigs, cattle and pigs. *Research in Veterinary Science*, **13**, 339–341.
- Sellers, R. F., Herniman, K. A., Leiper, J. W. and Planterose, D. N. (1973). The effect of a double-stranded RNA of fungal origin on the development of foot-and-mouth disease in pigs and calves. *Veterinary Record*, **93**, 90–93.
- Sellers, R. F. and Parker, J. (1969). Airborne excretion of foot-and-mouth disease virus. *Journal of Hygiene (London)*, **67**, 671–677.
- Shen, F., Chen, P. D., Walfield, A. M., Ye, J., House, J., Brown, F. and Wang, C. Y. (1999). Differentiation of convalescent animals from those vaccinated against foot-and-mouth disease by a peptide ELISA. *Vaccine*, **17**, 3039–3049.
- Snowdon, W. A. (1966). Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature*, **210**, 1079–1080.
- Sorensen, J. H., Jensen, C. O., Mikkelsen, T., Mackay, D. K. and Donaldson, A. I. (2001). Modelling the atmospheric dispersion of foot-and-mouth disease virus for emergency preparedness. *Physics Chemistry Earth*, **26**, 93–97.
- Sorensen, J. H., Mackay, D. K., Jensen, C. O. and Donaldson, A. I. (2000). An integrated model to predict the atmospheric spread of foot-and-mouth disease virus. *Epidemiology and Infection*, **124**, 577–590.
- Sorensen, K. J., Madsen, K. G., Madsen, E. S., Salt, J. S., Nqindi, J. and Mackay, D. K. (1998). Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Archives of Virology*, **143**, 1461–1476.
- Suryanarayana, V., Madanamohan, B., Bist, P., Natarajan, C. and Tratschin, J. D. (1999). Serotyping of foot-and-mouth disease virus by antigen capture reverse transcriptase/polymerase chain reaction. *Journal of Virological Methods*, **80**, 45–52.
- Sutmoller, P. and Cottral, G. E. (1967). Improved techniques for the detection of foot-and-mouth disease virus in carrier cattle. *Archiv für die Gesamte Virusforschung*, **21**, 170–177.
- Sutmoller, P. and Gaggero, A. (1965). Foot-and-mouth diseases carriers. *Veterinary Record*, **77**, 968–969.
- Sutmoller, P. and McVicar, J. W. (1968). Foot-and-mouth disease virus carrier studies at the Plum Island animal disease laboratory. *Session of the Research Group of the Standing Technical Committee, European Commission for the Control of Foot-and-Mouth Disease*, pp. 86–89.
- Sutmoller, P. and McVicar, J. W. (1973). Foot-and-mouth disease: growth of virus after conjunctival inoculation of cattle. Brief report. *Archiv für die Gesamte Virusforschung*, **43**, 284–287.
- Sutmoller, P. and McVicar, J. W. (1976). Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. *Journal of Hygiene (London)*, **77**, 235–243.
- Sutmoller, P., McVicar, J. W. and Cottral, G. E. (1968). The epizootiological importance of foot-and-mouth disease carriers. I. Experimentally produced foot-and-mouth disease carriers in susceptible and immune cattle. *Archiv für die Gesamte Virusforschung*, **23**, 227–235.
- Swenson, M. J. (1970). *Duke's Physiology of Domestic Animals*, 8th Edit, Cornell University Press, Ithaca, NY.
- Terpstra, C. (1972). Pathogenesis of foot-and-mouth disease in experimentally infected pigs. *Bulletin de l'Office International des Epizooties*, **77**, 859–874.

- Thomson, G. R., Bengis, R. G., Esterhuysen, J. J. and Pini, A. (1984). Maintenance mechanisms for foot-and-mouth disease virus in the Kruger national park and potential avenues for its escape into domestic animal populations. *Proceedings of the 13th World Congress in Buiatrics*, **13**, 33–38.
- Van Bakkum, J. G., Frenkel, H. S., Frederiks, H. H. J. and Frenkel, S. (1959a). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Bulletin de l'Office International des Épidémiologies*, **51**, 917–922.
- Van Bakkum, J. G., Frenkel, H. S., Frederiks, H. H. J. and Frenkel, S. (1959b). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift voor Diergeneeskunde*, **84**, 1159–1164.
- Vangrype, W. and De Clercq, K. (1996). Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. *Archives of Virology*, **141**, 331–344.
- Veletza, S. V., Nichols, K. V., Gross, I., Lu, H., Dynia, D. W. and Floros, J. (1992). Surfactant protein C: hormonal control of SP-C mRNA levels in vitro. *American Journal of Physiology and Lung Cellular and Molecular Physiology*, **262**, L684–L687.
- Williams, D. R. and Burrows, R. (1972). The growth of foot-and-mouth disease virus in organ cultures of bovine tissues. *Archiv für die Gesamte Virusforschung*, **37**, 145–159.
- Woodbury, E. L., Ilott, M. C., Brown, C. C. and Salt, J. S. (1995). Optimization of an in situ hybridization technique for the detection of foot-and-mouth disease virus in bovine tissues using the digoxigenin system. *Journal of Virological Methods*, **51**, 89–93.
- Yilma, T. (1980). Morphogenesis of vesiculation in foot-and-mouth disease. *American Journal of Veterinary Research*, **41**, 1537–1542.
- Yilma, T., McVicar, J. W. and Breese, S. S. (1978). Pre-lytic release of foot-and-mouth disease virus in cytoplasmic blebs. *Journal of General Virology*, **41**, 105–114.
- Young, S. L., Ho, Y.-S. and Silbajoris, R. A. (1991). Surfactant apoprotein in adult rat lung compartments is increased by dexamethasone. *American Journal of Physiology and Lung Cellular and Molecular Physiology*, **260**, L161–L167.
- Zhang, Z. D., Hutching, G., Kitching, P. and Alexandersen, S. (2002). The effects of gamma interferon on replication of foot-and-mouth disease virus in persistently infected bovine cells. *Archives of Virology*, **147**, 2157–2167.
- Zhang, Z. and Kitching, P. (2000). A sensitive method for the detection of foot and mouth disease virus by in situ hybridisation using biotin-labelled oligodeoxynucleotides and tyramide signal amplification. *Journal of Virological Methods*, **88**, 187–192.
- Zhang, Z. D. and Kitching, R. P. (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.

[Received, February 3rd, 2003]  
 [Accepted, April 11th, 2003]