The Pathogenesis of Foot-and-Mouth Disease I: Viral Pathways in Cattle

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Introduction

Foot-and-mouth disease virus (FMDV; family Picornaviridae; genus Aphthovirus) causes an acute disease of cloven-hoofed animals characterized by fever, lameness and vesicular lesions of the feet, tongue, snout and teats. These debilitating effects, rather than high mortality rates, are responsible for severe productivity losses associated with foot-and-mouth disease (FMD). The highly contagious nature of the virus and severity of economic impacts associated with the disease determine FMD’s status as the most important disease limiting trade of animals and animal products throughout the world.

The earliest recognition of the clinical entity of FMD is generally credited to Fracastorius’s observations in the 16th century (Fracastorius, 1546); however, the first step towards understanding the pathogenesis of FMD was Loeffler and Frosch’s landmark demonstration that the disease was caused by a filterable agent (i.e. virus) (Loeffler and Frosch, 1897). In the years since that discovery, investigation of the pathogenesis of FMD has been conducted in various manners and has been reviewed relatively recently (Grubman and Baxt, 2004; Alexandersen and Mowat, 2005). However, progress in understanding the mechanisms of this important disease has been impeded by two substantial obstacles: (i) the biosecurity requirements for working with the virus have limited research to only a handful of institutions worldwide and (ii) the seven serotypes and myriad strains of the virus have proven to often be as dissimilar as they are similar, thus limiting the ability to extrapolate findings from many studies to understanding of FMD in general.

It is generally accepted that FMDV spreads predominantly by direct or indirect contact with infected animals, their secretions or contaminated food products. It is also known that under certain circumstances, the virus travels over extensive distances to cause incursions at previously virus-free premises [reviewed in (Alexandersen et al., 2003b)]. Although airborne dissemination of infectious aerosols is often implicated, the contributory roles of humans (fomites), wildlife and waterborne spread are often not easily discerned. Conventional wisdom regarding FMD pathogenesis asserts that natural infection of cattle and sheep occurs via the respiratory route by aerosolized virus particles, whereas infection of domesticated animals is thought to be primarily by contact with infected animals or their secretions. More recently, experimental evidence has challenged the conventional wisdom of FMD pathogenesis and provided new insights into the mechanisms of this important disease.

Summary

In 1898, foot-and-mouth disease (FMD) earned a place in history as the first disease of animals shown to be caused by a virus. Yet, despite over a century of active investigation and elucidation of many aspects of FMD pathogenesis, critical knowledge about the virus–host interactions is still lacking. The aim of this review is to provide a comprehensive overview of FMD pathogenesis in cattle spanning from the earliest studies to recently acquired insights emphasizing works which describe animals infected by methodologies most closely resembling natural infection (predominantly aerosol or direct/indirect contact). The three basic phases of FMD pathogenesis in vivo will be dissected and characterized as: (i) pre-viraemia characterized by infection and replication at the primary replication site(s), (ii) sustained viraemia with generalization and vesiculation at secondary infection sites and (iii) post-viraemia/convalescence including resolution of clinical disease that may result in long-term persistent infection. Critical evaluation of the current status of understanding will be used to identify knowledge gaps to guide future research efforts.
Foot-and-Mouth Disease Pathogenesis in Cattle

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Stages of FMD Pathogenesis in Cattle

The terms ‘pre-viraemia’, ‘viraemia’ and ‘post-viraemia’ have generally accepted definitions in the description of stages of FMD pathogenesis. However, other terms such as ‘early’ and ‘persistent’ have been used variably in the published literature and have led to some confusion. While authors will, likely, continue to use terminology to suit their needs and preferences, for the purposes of this review, the stages of FMD pathogenesis are defined as:

1. Pre-viraemia: the period from when an animal is first infected with FMDV until virus is first detected within the intravascular (i.e. blood) compartment with a sustained and quantitatively increasing trend (determined by virus isolation (VI) or detection of viral RNA). Pre-viraemia may include a passive and transient presence of virus in the blood in the period immediately following first exposure to FMDV.

2. Establishment of viraemia: temporally occurs within pre-viraemia. However, because of the importance of this transition, it will be treated as a distinct event.

3. Viraemia: the period during which FMDV can be detected within the intravascular compartment with kinetics suggestive of active viral replication (determined by VI or detection of viral RNA). This period typically coincides with the clinical phase of disease. Viraemia may be undetectable in some infected animals even when they develop lesions at secondary sites of replication. However, if replication has occurred at secondary sites, viraemia must have occurred, and failure of detection in such instances may be attributed to insufficient frequency of sampling and/or inadequate detection sensitivity.

4. Post-viraemia: the period following viraemia starting with the first negative assay on blood (determined by VI or detection of viral RNA) which includes:
   (i) Resolution of clinical signs
   (ii) Short-term persistence of infectious virus, antigen and/or RNA in specific tissues
   (iii) Persistent infection (carrier state): the period after 28 dpi in which infectious FMDV may be detected on at least one of multiple oesophageal–pharyngeal (OP) samples (as defined by OIE)
   (iv) Chronic long-term sequelae including hirsutism, heat-intolerance (panting) and thyroid dysfunction, which have been reported in recovered cattle (discussed in companion article)

Pre-viraemia and Primary Sites of Infection in Cattle

FMD pathogenesis has been most thoroughly investigated in cattle, less so in swine and minimally in other species.
Yet despite several decades of investigation of the bovine pathogenesis, a clear consensus still does not exist regarding many basic aspects of virus–host interaction. Basic events of early infection, such as tissue sites and cellular characteristics of primary infection and replication, are not clearly established. Contradictions exist in the scientific literature that are likely due to multifactorial differences across studies including virus strains used, methods of virus detection, techniques used for animal exposure, tissues examined and variability amongst small numbers of experimental animals. Despite the relevance of all of these factors, strain-specific viral properties have likely created the most substantial challenge to generating a consensus understanding of the pathogenesis of FMD.

Amongst the myriad strains of FMDV, there surely are aspects of tropism and virulence which are strain specific; however, conclusions regarding these factors can not be adequately documented unless different strains are examined in vivo in studies in which all other experimental conditions remain constant. Such studies are rarely performed because of constraints of cost and logistics, and as such, the conclusions from most pathogenesis studies are only directly applicable to a single FMDV strain. Overall, upon examination of the complete breadth of the FMD pathogenesis literature, one is best advised to accept that many of the seemingly contradictory conclusions across studies may reflect intrinsic differences in experimental design.

Early investigators considered the natural routes of infection by FMDV to be via the upper gastrointestinal tract. However, as early as 1952, the susceptibility of cattle to respiratory tract inoculation was experimentally investigated (Henderson, 1952). Korn’s, 1957 work is often cited as the earliest demonstration that the respiratory tract provides the site of primary infection; however, in that work, the author cites two earlier studies that described infection of cattle via aerosol (Fredor, 1949) or intranasal deposition (Mohllmann, 1954). The remainder of the following discussion of pre-viraemia will focus upon contact and direct respiratory tract inoculation as these are considered to closely simulate natural exposure in cattle; however, it is acknowledged that cattle may sometimes be infected by direct transepithelial penetration and by ingestion, and this might result in different primary replication sites and distinct patterns of viral dissemination.

Korn (1957) infected cattle with FMDV by moist gauze deposition on the nasal planum and euthanized animals at various times after inoculation. By performing VI on various tissues, this work demonstrated that in pre-viraemic animals, FMDV was more frequently retrieved from nasal mucosae and nasopharynx than stratified squamous epithelial sites within the oral cavity. This work is also noteworthy in providing the only documentation of microvesiculation at a primary replication site (nasal mucosa).

Following Korn’s tissue-specific work, much of the investigation of FMD pathogenesis was conducted in studies utilizing VI from various clinical samples with an emphasis on OP fluid (‘probang samples’) and/or serum as a means of detecting ‘pharyngeal’ FMDV replication and viraemia, respectively (Burrows, 1968a; Sellers et al., 1968; McVicar et al., 1970; Burrows et al., 1971; McVicar and Sutmoller, 1976). Although these early works are informative, they clearly have limitations. The probang cup, while useful as a diagnostic instrument, provides a crude specimen for pathogenesis investigation. Although a VI-positive OP sample indicates the presence of infectious virus, it is insufficient to discern the origin of virus from amongst oesophagus, pharynx, oral cavity, lung or nasal cavity; each, individually, containing numerous distinct tissue and cell types. Regardless, in 1968, Burrows and colleagues demonstrated the presence of FMDV in OP samples prior to development of vesicles (Burrows, 1968a) and somewhat later confirmed pre-viraemic ‘pharyngeal’ replication by demonstrating that OP VI positivity occurred prior to detection of virus in blood subsequent to contact exposure (Sellers et al., 1968; Burrows et al., 1971). However, within approximately the same time period, Eskildsen (1969) brought into question the notion that VI-positive probang samples were necessarily indicative of pharyngeal FMDV replication by demonstrating that deposition of FMDV directly into the lung via tracheostomy consistently resulted in generalized disease. Thus, the relative contributions of upper and lower respiratory tract to FMD pathogenesis were already in question.

The pre-viraemic viral dynamics in the bovine respiratory tract were more precisely defined in subsequent experiments which demonstrated that primary replication of FMDV could be detected in OP fluid within 2–6 h of intranasal deposition depending upon viral strain and dose administered (McVicar et al., 1970; McVicar and Sutmoller, 1976). These experiments also demonstrated that the onset of viraemia occurred at or near the peak of FMDV detection from OP samples [16–72 h post-inoculation (hpi)] and that the lag (eclipse) time between inoculation and OP positivity was inversely related to the FMDV dose inoculated.

In 1981, the first comprehensive examination of distribution of FMDV in numerous distinct tissues during acute infection of cattle was reported (Burrows et al., 1981). By returning to the strategy of dissecting and screening tissues individually, this work provided compelling evidence that in the pre-viraemic phase, FMDV-positive OP samples likely represented pharyngeally
replicated FMDV. Additionally, the use of several routes of exposure and three different viruses broadened the implications of this work. Overall, amongst pre-viraemic cattle, dorsal soft palate, pharynx (precise region not indicated) and retropharyngeal lymph node were the tissues most frequently found to be VI positive and had the highest mean FMDV titres. These experiments found other tissues including lungs, tonsils and nasal mucosae to be involved in pre-viraemic FMD with lower frequency and at generally lower viral titres.

In theory, microscopic localization of FMDV during pre-viraemic infection should provide the most definitive information regarding the primary site(s) of infection; however, the combinatorial data from a handful of studies is less than completely congruous. Microscopic localization of pre-viraemic FMDV has been performed by *in situ* hybridization (ISH) (Brown et al., 1992, 1996; Hofner, 1995) and immunohistochemistry (Arzt et al., 2010; Pacheco et al., 2010). Brown and colleagues inoculated cattle using an improvised aerosol chamber and examined several tissues by ISH at various times post-exposure. The overall conclusion was that the lungs were the most likely primary infection site based on localization of FMDV RNA to respiratory bronchioles in (presumed) pre-viraemic steers. However, viewed retrospectively, both works from this group have perplexing findings. In the earlier work (Brown et al., 1992), the strongest ISH signal at 6 hpi was detected at the lesion predilection sites of the feet with weaker signal seen in tongue and carpus (all unexpected in pre-viraemic animals). This could be explained either by the occurrence of a transient, low grade, undetected viraemia or by pre-existing (non-FMD-related) lesions that became FMDV contaminated at the time of aerosol inoculation. The latter hypothesis is unlikely because the finding was similarly repeated in several animals within the study, and the former cannot be addressed as blood was not collected from the animals euthanized at early time points (discussed further below). Additionally, it is unfortunate that tissues of the pharynx and nasal cavity were not examined within that study (Brown et al., 1992) to address the earlier indications that such tissues were important primary replication sites (Korn, 1957; Burrows et al., 1981). The later work from this group did examine the soft palate that was FMDV ISH negative at 24 hpi; however, only a single (presumed) pre-viraemic animal was included for each virus used in this study (Brown et al., 1996).

Two recent works have investigated early pathogenesis in an aerosol inoculation model using trimal (VI, rRT-PCR, immunomicroscopy) detection systems for FMDV (Arzt et al., 2010; Pacheco et al., 2010). These studies screened a large quantity of tissues from animals spanning the pre-viraemic period; however, the works have the similar limitation of examining only a single serotype of FMDV. These authors demonstrated that within this system, bovine FMDV infection initiated (6 hpi) at the crypt regions of the follicle-associated epithelia at mucosa-associated lymphoid tissue (MALT) regions of the nasopharynx (Figs 1 and 2). Shortly thereafter (12 hpi), FMDV replication (i.e. immunoreactivity for non-structural proteins) was detected within the pulmonary alveolar septa. At both pharyngeal and pulmonary sites, FMDV colocalized with cytokeratin indicating that the infected cells were of epithelial histogenesis. Furthermore, these works demonstrated a trend that as viraemia approached, FMDV became more prominent within the lungs and less apparent in the pharyngeal tissues as determined by all three modalities of detection. It is noteworthy that lymph nodes from pre-viraemic cattle were nearly uniformly FMDV negative.

Lastly, various studies have directly demonstrated or indirectly suggested that FMDV may enter the systemic circulation and disseminate to distant sites during the pre-viraemic period. This leads to the semantic issue of whether it is appropriate to define primary and secondary viraemias in the pathogenesis of FMD. Distinct primary viraemia has been identified in the pathogenesis of morbilliviruses (Auwaerter et al., 1999; von Messling et al., 2004) and herpesviruses (Cohen, et al., 2007). There are sporadic reports of detection of transient, low titre (trace) viraemia in cattle following contact infection or exposure to FMDV aerosols (Hofner, 1995; McVicar and Eisner, 1983; Sutmoller and McVicar, 1976b). Additionally, in calves exposed to aerosolized virus, the detection of FMDV RNA in pedal and oral epithelia at six hpi, before the onset of ‘true’ viraemia and clinical signs is best explained by haematogenous spread (Brown et al., 1992). FMDV has also been isolated from the pharynx in cattle as early as four hours after virus was instilled into mammary tissue, before the onset of viraemia (Burrows et al., 1971). It is feasible that low titres of cell-free or cell-associated virus were present in the circulation in these animals but were undetectable by current methodologies because of the large volume of the circulatory system (Sutmoller and McVicar, 1976a). Overall, the consistency and the mechanisms by which this putative primary low-level viraemia occurs remain to be elucidated, and the significance to overall understanding of FMD pathogenesis remains unknown.

Establishment of Viraemia in Cattle

Understanding the mechanisms by which FMDV enters the systemic circulation and maintains high-titre viraemia is of critical importance to basic scientific understanding of the pathogenesis, but also to control efforts. Effective abrogation of viraemia markedly decreases severity of
Fig. 1. Tissue-specific detection of foot-and-mouth disease virus (FMDV) or viral RNA by virus isolation (VI) or rRT-PCR in pre-viraemic steers inoculated via aerosol with \(10^7\) BID<sub>50</sub> of FMDV-O1-Manisa 3–24 h prior. Only epithelia of the nasopharynx and larynx occupy the highest stratum of 80–100% indicating these tissues as the most consistent sites of primary infection. Prevalence values were calculated as number of animals in which a tissue was determined positive/number of animals in which that tissue was assayed. Inclusion criterion was negative VI on serum at the time of euthanasia. Data adapted from Arzt et al., Veterinary Pathology 2010.

Fig. 2. Primary infection of epithelial cells of the nasopharynx is the earliest microscopically detectable event in foot-and-mouth disease in cattle. Multichannel immunofluorescent technique was applied to bovine dorsal soft palate 12 h after aerosol inoculation with \(10^7\) BID<sub>50</sub> of foot-and-mouth disease virus (FMDV)-O1-Manisa. Regional orange staining represents colocalization of signal from FMDV capsid (red) and pancytokeratin (green). CD11c-positive (light blue) and MHC-II-positive (dark blue) cells are present individually and in clusters in close proximity to, but with no specific relationship to FMDV-positive cells. Scale bar, 100 µm.
clinical disease and extent of shedding, thus the economic impact of incursion into naïve herds or flocks. Indeed, current vaccines likely function by preventing viraemia not primary infection. Despite the importance of this issue, very few pathogenesis studies have specifically addressed how viraemia is established, and the conventional wisdom on the subject is derived almost entirely from speculation. To suggest that an organ or tissue may serve as a ‘portal’ for establishment of viraemia, certain requirements should be met individually or, ideally, in combination: (1) FMDV should be detected in that tissue prior to detection of viraemia, (2) experimental exposure of that tissue (in isolation) should be shown to result in viraemia and (3) a cellular/molecular mechanism for movement of FMDV from the interstitium to the intravascular compartment should be demonstrated (or sensibly projected).

Although requisite 3 is the most elusive at present given the incomplete elucidation of FMDV–host molecular interactions in vivo (discussed in the companion manuscript), several studies have met requisites 1 and 2. As discussed earlier (previraemia section), several studies have demonstrated the presence of FMDV in the respiratory tract prior to viraemia (Arzt et al., 2010; Burrows, 1968a; Burrows et al., 1981; Korn, 1957; McVicar et al., 1970; Sutmoller and McVicar, 1976b). However, defining the viraemia portal (upper versus lower respiratory tract) is complicated by the fact that these putative sites are interconnected, and in live animals, air flow (and hence aerogenous FMDV) passes freely between the external environment, nasal cavity, pharynx and all levels of lungs. This complexity was elegantly addressed by Sutmoller and McVicar (1976b) by placement of tracheostomy tubes in steers as a means of isolating the upper and lower respiratory tracts for examination of viral dissemination under variable exposure conditions. By demonstrating similar FMDV kinetics in blood between tracheostomized cattle inoculated either intranasally or by contact, this work concluded that either the upper or lower respiratory tract may individually serve as portals of FMDV entrance to the systemic circulation. Further evidence supporting the importance of the nasopharynx in establishing viraemia came from Burrows’ work, which demonstrated that lungs of aerogenously infected cattle were often (but not exclusively) VI negative unless the animals were viraemic; this suggested that the virus had to reach some other site (presumably nasopharynx) to establish viraemia which then could be detected in lung. However, more recent work has shown that in the period in which viraemia is established, substantially greater quantities of FMDV are detectable in the lungs when compared to the pharynx suggesting a pulmonary portal (Arzt et al., 2010). This finding has been consist-
may be extensive replication in the palatine tonsils (Fig. 3) and lungs (Arzt et al., 2010).

The anatomical and cellular source of the high titres of virus detected in the blood during viraemia remains one of the important knowledge gaps regarding FMD. Typically, only vesicles have greater quantities of FMDV than viraemic blood which suggests a vesicular source, except that viraemia can precede vesicular lesions for several hours or days and in some cases viraemia is present in the absence of grossly detected vesicular lesions. Also, it is unlikely that several minute vesicles could maintain the high viraemic titres of FMDV given the large bovine blood volume. However, microscopic vesicles have been described in the tongue and haired skin of cattle in the absence of macroscopic lesions (Seibold, 1963; Gailiunas, 1968; Yilma, 1980). Despite the absence of gross lesions, it has been shown that bovine haired skin can contain relatively high titres of virus, approaching but not exceeding the amount detected in the blood at the peak of viraemia (Gailiunas and Cottral, 1966). Thus, virus replicating within non-lesional epithelial sites (i.e. skin) could contribute to the high-titre viraemia detected in animals in the absence of macroscopic lesions (Brown et al., 1995; Murphy et al., 2010). Additionally, it has recently been suggested that FMDV replication in the lungs of cattle may substantially contribute to maintenance of high-titre viraemia (Arzt et al., 2010). Detection of high quantities of viral RNA, antigen and infectious virus in pulmonary tissues starting at the onset of viraemia combined with the large mass of the lungs supports this notion.

Several studies have reported tissue-specific viral loads in viraemic cattle (Burrows et al., 1981; Zhang and Alexandersen, 2004; Arzt et al., 2010). However, such data must be reviewed cautiously as it is impossible to accurately determine the contribution to a tissue’s ‘viral load’ from bloodborne virus. The common approach to correct this complication by subtracting the blood titre from the tissue titre is an imperfect solution as it falsely assumes similar blood volumes in all tissues examined. Bearing this limitation in mind, it is still noteworthy that the highest viral loads reported in these studies (excluding vesicles) were lymph nodes and myocardium (Burrows et al., 1981), lungs (Arzt et al., 2010) and vesicle-free lesion predilection sites (Zhang and Alexandersen, 2004).

There is limited and contradictory evidence regarding replication or transport of FMDV in peripheral blood mononuclear cells (PBMC) in cattle (Alexandersen et al., 2002a; Zhang and Alexandersen, 2004). By contrast, a transient lymphopenia has been detected early after infection in swine which may be a consequence of infection of T cells (Bautista et al., 2003; Diaz-San Segundo et al., 2006). However, the susceptibility of porcine PBMC to FMDV infection may be dependent on the serotype of challenge virus, for example; PBMC isolated from serotype C-infected swine were shown to be infected during...
viraemia, coinciding with depletion of T cells in lymph nodes and the spleen (Diaz-San Segundo et al., 2006). In contrast, PBMC isolated from serotype O-infected swine were not infected, and the transient lymphopenia may be the consequence of recruitment of lymphocytes from blood to sites of infection and inflammation (Bautista et al., 2003; Toka et al., 2009). There are no reports in the literature documenting significant lymphopenia or generalized immunosuppression in cattle as a consequence of FMDV infection (Juleff et al., 2009). Although it is apparent that FMDV can interact with bovine lymphocyte populations in vitro (Harwood et al., 2008; Joshi et al., 2009; Summerfield et al., 2009), the role of these interactions during viraemia, and on FMD pathogenesis in general, remains to be determined.

Dendritic cells (DCs) have a central role in the induction of innate and adaptive immune responses, yet their role in FMD pathogenesis is poorly understood. The various subpopulations of DCs and their precursors are functionally unusual in their ability to move from tissues into the (lymphatic) vasculature and, additionally, from the blood into tissues. It has been demonstrated that the presence of anti-FMDV antibodies may lead to a shift in affinity and uptake of antibody-opsinized virus by Fc receptor–expressing cells such as DCs (Summerfield et al., 2009). This shift in affinity may be responsible in part for the early localization of FMDV to lymphoid follicles, which develop into germinal centres (GCs) following antigen exposure. FMDV has been detected in mandibular lymph node follicles as early as 3 days post-intradermal challenge (Juleff et al., 2008). GCs are an important component of the humoral immune response, and B cells in the GC macroenvironment undergo intense proliferation, selection, maturation and death during antibody responses (McHeyzer-Williams and McHeyzer-Williams, 2005). The precise influence of FMDV localization within GCs on the immune response remains to be determined; however, it is likely that at this stage critical events transpire which initiate the early phase of the adaptive immune response.

An effective immune response against FMDV, leading to clearance of viraemia and tissue viral loads, is characterized by the rapid induction of specific antibody and is thought to be dependent on the interaction between antibody–virus complexes and the phagocytic cells of the reticuloendothelial system (McCullough et al., 1986, 1988, 1992; Juleff et al., 2009). These interactions may account for the accumulation of FMDV in the spleen, liver and lymph nodes during viraemia reported in some studies (Zhang and Alexandersen, 2004). Although FMDV structural and non-structural proteins have been detected in lymph nodes of cattle during viraemia, FMDV proteins have not been detected in the spleen or liver, and it is not clear if FMDV undergoes productive replication at these sites (Juleff et al., 2008).

**Post-Viraemia**

It is well established that in the convalescent period, subsequent to clearance of viraemia, FMDV continues to be present in very high titres at the lesion predilection sites. Additionally, after clearance of virus from lesion sites, FMDV persists in certain tissues for prolonged times and a subset of FMDV-infected ruminants develop chronic asymptomatic infection referred to as persistence or the carrier state (Alexandersen et al., 2002a; Salt, 2004a). Although various trends have been described regarding tissues affected, species susceptibility and viral genomic alterations associated with persistence, much remains unknown. A noteworthy knowledge gap is the poor understanding of the extent of the threat posed by carriers to naïve animals. However, global trade policy is intimately tied to FMD persistence and two inescapable realities are (i) that the concern of transmission from asymptomatic carriers is the main reason why FMD-free nations restrict imports of live hoof stock from enzootic regions or regions with freedom with vaccination and (ii) one of the main reasons that FMD-free nations depopulate when confronted with FMDV incursion is because of failure of vaccination to prevent the carrier state.

The historic recognition of FMDV persistence in ruminants has been reviewed extensively (Salt, 1993; Alexandersen et al., 2002a, 2003b; Grubman and Baxt, 2004). Briefly, the recovery of infectious FMDV from convalescent cattle was first convincingly demonstrated by Van Bekkum et al. (1959). Reports of similar findings from various investigators established the notion that FMD persistence (defined as recovery of live virus at more than 28 dpi) is a common sequel to infection of ruminants and that roughly 50% (with substantial variability across studies) of ruminants become persistent carriers for a variable period of time. Another landmark was Burrows’ demonstration of tissue-specific localization of persistence with dorsal soft palate and dorsal pharynx implicated as the most frequent sites of recovery of FMDV from post-viraemic cattle (Burrows, 1966, 1968b). Yet, the anatomical and cellular sites where FMDV persists and the origin of virus detected by probang sampling still remain incompletely elucidated. Burrows had additionally recovered FMDV from several other tissues of carrier animals including the oesophagus, ventral soft palate, pharynx, glosso-epiglottic space and tonsillar sinuses. These early studies were somewhat limited in that they detected FMDV by VI, a technique which may be compromised in carrier animals by the presence of high titres of neutralizing antibody. Work by Donn et al. (1994) highlighted the
limitations of applying conventional VI techniques for detecting the carrier state in tissue samples and the benefits of detecting viral RNA by the polymerase chain reaction method. In that study, viral RNA was detected in the tonsil, ventral and dorsal soft palate and cranial oesophagus of contact challenged cattle; however, all the tonsillar and oesophageal samples were negative by VI. Although this may indicate a greater sensitivity of RT-PCR relative to VI, the molecular approach to detection has a distinct limitation in that it gives no indication whether or not detected RNA is associated with infectious virus.

More recently, detailed time course experiments have characterized clearance of FMDV RNA from tissues during the immediate post-acute period in cattle (Zhang and Alexandersen, 2004). This work demonstrated that viral RNA was detectable in various tissues for up to two days after cessation of viraemia, but was largely cleared from most tissues of cattle at 14 days post-challenge. However, the same study demonstrated FMDV RNA in pharyngeal tissues (carrier and non-carriers) and lymph nodes (carriers only) up to 72 dpi. Notably, only one tissue (dorsal soft palate) contained viral RNA in every animal from which the probang specimen was positive by VI. This correlation strongly suggested a dorsal palatal source of virus detected via probang. Other works have similarly described that viral RNA is detectable in pharyngeal tissues beyond 28 dpi (Salt, 1993; Alexandersen et al., 2002a). Similarly, in sheep, viral RNA has been detected in tonsil, dorsal soft palate and nasopharynx up to 43 days post-needle or contact challenge (Horsington and Zhang, 2007). A recent study in sheep showed that by 10 dpi, the only tissues where FMDV still replicated were the tonsil and soft palate (Ryan et al., 2008).

Microscopy studies utilizing ISH in bovine tissues have provided further support for the importance of pharyngeal tissue in the convalescent (5–17 dpi) (Prato Murphy et al., 1999) and persistent (42–82 dpi) (Zhang and Kitching, 2001) periods. Both of these studies described intraepithelial localization of FMDV RNA within dorsal and ventral soft palate and pharynx with the strongest ISH signal detected in the basal and deep spinous regions. Although the phenotypes of infected cells were not investigated in these works, the signal distribution was most consistent with epithelial cells. Strong ISH signal was also identified within the palatine tonsil at 14 dpi with a signal distribution suggestive of lymphoid follicle association (Prato Murphy et al., 1999). Microscopic localization of FMDV antigens in these regions from carrier animals has not been published previously; however, recent work has suggested that FMDV structural and non-structural antigens are present in pharyngeal tissues with a more limited distribution compared to that described by ISH (Fig. 4, Pacheco et al., manuscript in progress). The detection of non-structural proteins supports the concept that virus is replicating at these sites during persistence. Yet, the histogenesis of cells involved remains elusive with morphologic and phenotypic evidence suggesting roles for dendritic cells and epithelial cells.

Additional insights on tissues involved in persistence has come from a recent study that examined pharyngeal tissue samples harvested from cattle 38 days post-contact challenge using a combination of different techniques to detect viral RNA and proteins (Juleff et al., 2008). These investigators readily detected FMDV genome, using laser capture microdissection and qRT-PCR, in GCs within the dorsal soft palate, pharyngeal tonsil, palatine tonsil, lateral retropharyngeal lymph node and mandibular lymph node. In contrast to earlier ISH studies, viral RNA was not identified within the overlying epithelia. These findings were confirmed by ISH studies and by immunohistochemistry using a monoclonal antibody specific for conformational, non-neutralizing epitopes on the FMDV capsid. FMDV capsid antigen (but not non-structural proteins) was detected in mandibular lymph node GCs of 22 animals examined between 29 and 38 days post-contact infection, irrespective of the carrier status at the time of euthanasia. These novel findings have provided evidence for a previously undescribed stage of FMD in cattle in which it is presumed that the virus persists in a non-replicative form. Because this condition was observed in carriers and non-carriers, it was proposed that it may occur as a common sequel to infection regardless of whether virus replication was persisting at other sites. However, it remains to be elucidated whether FMDV detected in lymphoid tissue GCs is replication competent. Furthermore, it is unclear if these virus depots contribute to viral repopulation of other cells in the pharynx with subsequent replication and release of FMDV (i.e. probang-positive carriers). Future studies in a number of species, including the African buffalo and possibly appropriate mouse models, will answer these questions.

Various mechanisms have been proposed to explain the establishment and maintenance of FMDV persistence [reviewed in (Alexandersen et al., 2002a; Salt, 2004b)]; however, further elucidation is clearly required. The hypothesis that immune mechanisms play a role in persistent infection in ruminants is supported by the early observations that infected vaccinated cattle become persistently infected more consistently than unvaccinated ones (McVicar and Sutmoller, 1969) and observations by Ilott et al. (1997) that dexamethasone treatment decreases viral shedding from persistent cattle. One study compared cytokine and toll-like receptor RNA levels in dorsal soft palate lymphoid tissues between carrier and non-carrier cattle at 64 dpi with only TNF-α having significantly elevated levels in persistently infected animals (P = 0.027, n = 2) (Zhang et al., 2006). By contrast, all other genes
investigated (IFN, β, γ, IL1a, 2, TLR-3, 4) had similar expression between carrier and non-carrier tissues. In a separate report, it was described that IFN-γ treatment of persistently FMDV-infected cells resulted in marked decrease in detection of viral antigens and RNA suggesting that this cytokine may play a role in clearance of infection in vivo (Zhang et al., 2002).

Few studies have demonstrated that FMDV capsid protein mutations and associated antigenic variation occur during persistent infection in vitro (Diez et al., 1990; Martin Hernandez et al., 1994; Holguin et al., 1997) and in vivo (Gebauer et al., 1988; Salt, 1993; Jangra et al., 2005). It has been suggested that the failure of high levels of IgA in OP fluid to clear FMDV in carrier cattle may be partly due to such antigenic variation. Recently, it was demonstrated that a substitution change in the B-C loop of VP2 may be associated with persistent FMDV infection in cattle (Horsington and Zhang, 2007). Antigenic sites of FMDV VP2 are of high immunological importance, and mutations within these sites have been shown to affect antigenicity (Kitson et al., 1990). Although such works raise intriguing questions regarding mechanisms of persistence, much work remains to be performed.

**Conclusions**

Cure, prevention and eradication are the ultimate goals of the study of any disease. Even basic research feeds into a cumulative effort directed towards disease control and eradication. Considering these goals in the context of FMD, the modern investigator may take solace from the accrual of some major accomplishments over the last 100 years; however, much work clearly remains. Various high priority knowledge gaps have been mentioned throughout the preceding sections and are reiterated in Table 1; the bridging of these gaps will, ultimately, contribute to FMD control and eradication.

It may be asserted that the biggest problem overshadowing all of FMD research is that (based upon recent history) incursion of FMDV into FMD-free nations still results in massive depopulations of uninfected but ‘high-risk’ animals. This practice is based more upon policy than science and is closely related to the inability of currently available vaccines to prevent primary infection and persistence. The rationales behind use of depopulation as the means of control are (i) eliminating any infected, acute shedders of large quantities of virus (or susceptible...
Table 1. High priority knowledge gaps in understanding of FMD pathogenesis

<table>
<thead>
<tr>
<th>Question</th>
<th>Details</th>
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<tbody>
<tr>
<td>What are the sites of primary infection (tissue and cellular understanding; multiple serotypes/strains and host species)?</td>
<td>foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<tr>
<td>What are the host (cellular and molecular) determinants of tropism beyond integrins?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>What innate and adaptive immune factors contribute to tropism?</td>
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<tr>
<td>Which innate and adaptive immune factors are subverted by FMDV and by what mechanisms?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>What are the roles of dendritic cells, NK cells and γδ-T cells during the various stages of infection?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>What are the mechanisms of shedding of virus into secretions and naturally generated aerosols?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<tr>
<td>How can understanding of tropism and primary infection be manipulated to generate ‘rationally designed’ vaccines?</td>
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<tr>
<td>What are the sites and mechanisms of establishment of viraemia?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<tr>
<td>How common is primary, passive viraemia and what is the relevance to pathogenesis?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>What are the sites of replication responsible for maintenance of viraemia?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
</tr>
<tr>
<td>What are the viral and host determinants of establishing persistence at the species and individual animal levels? (How) does vaccination affect persistence?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
</tr>
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<td>What are the anatomical and cellular sites of persistence in different ruminant species?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>Why are ruminants, but not swine susceptible to persistent FMDV infection?</td>
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<td>To what extent are persistently infected ruminants a threat to naïve animals?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
</tr>
<tr>
<td>What unique pathogenesis events occur in gravid females and embryo/foetuses?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<tr>
<td>What are the specific virus–host interactions which determine (cardio)myotropism?</td>
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<td>What are the most appropriate challenge models in the different species to study FMDV pathogenesis?</td>
<td>Foremost amongst numerous potential explanations are the exceedingly broad categories of unique cellular characteristics and immunological (innate and/or adaptive) factors. A more thorough understanding of these processes ultimately may lead to effective modulation of such cellular and immune responses for the benefit of preventing or resolving infection.</td>
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<td>Does cell cycle status have a role in determining permissiveness to FMDV replication and persistence?</td>
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FMDV, foot-and-mouth disease virus; FMD, foot-and-mouth disease.
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References


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