Review

Skin as a potential source of infectious foot and mouth disease aerosols

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This review examines whether exfoliated, virus-infected animal skin cells could be an important source of infectious foot and mouth disease virus (FMDV) aerosols. Infectious material rafting on skin cell aerosols is an established means of transmitting other diseases. The evidence for a similar mechanism for FMDV is: (i) FMDV is trophic for animal skin and FMDV epidermis titres are high, even in macroscopically normal skin; (ii) estimates for FMDV skin cell aerosol emissions appear consistent with measured aerosol emission rates and are orders of magnitude larger than the minimum infectious dose; (iii) the timing of infectious FMDV aerosol emissions is consistent with the timing of high FMDV skin concentrations; (iv) measured FMDV aerosol sizes are consistent with skin cell aerosols; and (v) FMDV stability in natural aerosols is consistent with that expected for skin cell aerosols. While these findings support the hypothesis, this review is insufficient, in and of itself, to prove the hypothesis and specific follow-on experiments are proposed. If this hypothesis is validated, (i) new FMDV detection, management and decontamination approaches could be developed and (ii) the relevance of skin cells to the spread of viral disease may need to be reassessed as skin cells may protect viruses against otherwise adverse environmental conditions.

Keywords: epidermal desquamation; virus excretion; aerosol emission; airborne transmission; epidemiology; foot and mouth disease

1. INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease capable of causing widespread epidemics among livestock. It has a major economic impact when outbreaks occur in countries previously free from disease. The foot and mouth disease virus (FMDV) is virulent and has multiple known routes of transmission. These include direct contact (e.g. viral entry through mucous membranes, cuts or abrasions during animal-to-animal contact), indirect contact (e.g. fomites), ingestion (e.g. contaminated feed) and the respiratory or airborne pathway (e.g. the inhalation of infectious aerosols) [1]. The airborne pathway is suspected to play a key role in some outbreaks by causing disease 'sparks' (i.e. disease spread to regions remote from a primary infection site) [2,3]. If not detected in a timely fashion, such sparks can lead to major outbreaks. For example, the widespread dissemination of FMDV during the catastrophic 2001 UK outbreak was thought to be due to the inadvertent transport of animals with unrecognized FMDV infection from a Prestwick farm to areas previously free of FMDV [4].

Like other viral diseases with an airborne transmission pathway, the source of infectious FMDV aerosols is generally considered to be virus exhaled from the respiratory system [1]. However, while whole-animal FMDV-infected aerosols have been extensively characterized, a literature search identified only one study [5] that directly demonstrated

that the respiratory system was a source of airborne FMDV.[1] It is also noteworthy that one study [6] measured significant emissions of infectious FMD aerosol when swine were placed in looseboxes *after being killed*—when, presumably, all respiratory release of virus had ceased.

This review examines the possibility that FMDV-infected skin cells may be an additional source of infectious FMD aerosols. Early researchers did previously raise the possibility that airborne FMDV-infected skin cells might be important in disease transmission [6–8]; however, this possibility was never systematically investigated. In contrast, *respiratory* mucosal epithelial cells are known to be a primary site of initial infection (pharynx), a main virus amplification site (mouth) and the site of persistent infection in carrier ruminants (pharynx) [1,9]. It is also known that FMDV is often found in oral–pharyngeal fluids containing cellular material while samples without cellular material are typically FMDV negative [1,9]. Collectively, these observations suggest that FMDV-infected, respiratory mucosal epithelial cells shed into respiratory fluids may contribute to respiratory emissions of FMDV aerosols.

Mammalian skin actively sheds a significant number of skin cells (10^6 to 10^8 per day) into the environment [10–12] and skin cells have been observed to comprise a significant fraction (1–10%) of measured indoor and outdoor[2] aerosols and indoor dust [13–16]. Bacteria, yeast, fungi and viruses are present on the surface of skin cells (e.g. [17] and references within). When these skin cells mature and naturally exfoliate, the infectious material can become airborne (electronic supplementary material, Particle Suspension Mechanisms), travel to new hosts and cause infection when inhaled or deposited directly onto the skin of the

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new host [10,18–22]. This mechanism is believed to be a significant source of bacterial infection for surgical procedures and other nosocomial infections [10,18]. Transmission of viral disease via the inhalation of infectious skin cells is less well studied, but may be documented in at least one case (electronic supplementary material, Other Viral Diseases).

The purpose of the current study is to systematically review published data relevant to the hypothesis that skin cells could be a source of infectious FMDV aerosols. Estimates are provided for (i) skin cell shedding rates, (ii) FMD skin concentrations and (iii) the shedding rate of FMDV-infected skin cells. In addition, the expected characteristics of an infectious FMDV skin cell aerosol source are placed in context with known experimental data. These include measurements of whole-animal FMDV aerosol emissions in relation to timing, aerosol stability, aerosol size and magnitude. Suggestions for future experiments are provided.

2. ESTIMATING THE SHEDDING RATE OF FOOT AND MOUTH DISEASE VIRUS-INFECTED SKIN CELLS

(a) Animal skin cell shedding rate

As part of the normal skin growth cycle, mammalian skin cells normally move progressively from basal cells (stratum basale) within the epidermal layer of the skin outward to the stratum corneum, where old skin cells then exfoliate into the environment. In adult humans (the most studied species with respect to airborne skin cell emissions), healthy skin typically sheds one cell layer per day. Exfoliated skin cells are typically shed as individual hexagonal plates, 25 µm on a side and 0.1–0.5 µm thick [11,12]. Mature skin cells (corneocytes) can become airborne by air moving across the skin surface [23] (see also electronic supplementary material, Particle Suspension Mechanisms); however, emissions over a short period of time can significantly increase with mechanical abrasion (e.g. rubbing of clothes or body parts [24]), physical activity [25,26] and/or washing [27]. Exfoliated skin cells in settled dust may become re-aerosolized by human (animal) activity [13,20,21] (see also electronic supplementary material, Particle Suspension Mechanisms). The median aerodynamic diameter[3] of human skin cells is approximately 14 µm. In fresh [25,28] and environmentally processed [16] emissions, skin cells are observed at both smaller and larger sizes—although the size distribution of aerosols derived from skin cells is not precisely defined in the current literature.

Human skin bears many similarities to the skin of domestic animals that have been documented to emit airborne FMDV (e.g. swine, cattle and sheep) [29–34]. The similarities include general structure, skin cell size and epidermial cell turnover time. Based on these similarities, swine, cattle and sheep can be expected to normally shed one layer of skin cells per day. Considering an animal's skin surface area, a nominal epidermis thickness[4] of 100 µm and an assumed skin density of 1 g cm^{-3}, the estimated mass of epidermal material shed per day is 2 g for swine and sheep and 10 g for cattle.[5]

(b) Animal skin foot and mouth disease virus concentrations

While not a typical site for the initial FMDV infection, the skin is a major viral replication site in most animals studied [1,8,35–39]. Table 1 and electronic supplementary material, table S1 summarize the available literature on swine, cattle and sheep FMDV skin concentrations for the day on which infectious FMDV skin concentrations are highest.[6] Infectious FMDV concentrations in skin on the body surface are presented for both clinically abnormal external (non-oral) skin lesion material (typically foot lesions) and in macroscopically normal (but infected) skin. As FMDV skin concentrations are known to vary by body region, measurement data are presented for both the trunk and extremity measurements.

FMDV is well known to be present in the macroscopic skin lesions characteristic of clinically active disease. The rupture of these macroscopic skin lesions, with the subsequent release of FMDV-infected cell cytoplasm onto the surface of the skin followed by exfoliation of the infected skin cells, is one pathway whereby FMDV could become aerosolized[7] (i.e. FMDV 'rafting' on the outside of airborne skin cells) [37,42,45].

There is also the possibility that FMDV-infected skin cells from skin appearing clinically normal could be a source for FMDV aerosol and disease transmission. All seven antigenic types of FMDV have been observed in the normal skin of infected animals (i.e. skin without clinically obvious, macroscopic lesions), albeit at a lower concentration than in lesional material. Brown *et al.* [37,46] and Gailiunas [39] observed microscopic lesions to be present just below the stratum corneum in some (but not all) of the FMDV-positive, clinically normal skin samples that were examined.

Within the skin itself, FMDV concentrations are highest (by several orders of magnitude) within the epidermis [37,39]. *In situ* hybridization and immunofluorescence studies indicate that the initial FMDV replication site is located in the deeper basal layers of the epidermis (basal cells proper or the stratum spinosum layer just above) and that FMDV-laden cells migrate outward towards the skin surface. There is no evidence of active virus replication in the stratum corneum [37,42,45,46]. Brown *et al.* [37] reported FMDV present within the cell cytoplasm of all epidermal skin layers in macroscopically normal epidermis. Other studies [45,46] have not observed the FMDV signal in the intact, non-lesional stratum corneum. There are no known studies of the infectivity of the stratum corneum in animal skin.

(c) Peak foot and mouth disease virus-infected skin cell shedding rates

The peak FMDV skin cell shedding rate is estimated by multiplying the skin cell shedding rate by the peak FMDV skin concentrations (§2a,b). This calculation yields a peak FMDV skin cell shedding rate of approximately 10^6 TCID$_{50}$ per animal per day for swine and cattle, respectively, based on non-lesional FMDV skin concentration measurements.[8] This estimate is approximate and does not include the contributions of infected FMDV skin cells derived from lesional material—which contains FMDV concentration orders of magnitude higher than non-lesional skin. It also does not include the contribution of skin externally contaminated with infectious FMDV. Both of these mechanisms would be expected to increase the net infectious skin cell shedding rate. The fraction of shed skin cells that are aerosolized, either initially or at a later time, is likewise unknown, but the FMDV-infected skin cell aerosol emission rate would be less than the skin cell shed rate estimated in this

Table 1. Peak external skin FMDV concentrations. AVE, average of values.

sample location	cattle study	cattle average FMDV skin concentration ($\log_{10}(\text{TCID}_{50})\ g^{-1}$)[a]	cattle no. measurements	swine study	swine average FMDV skin concentration ($\log_{10}(\text{TCID}_{50})\ g^{-1}$)[a]	swine no. measurements	sheep study	sheep average FMDV skin concentration ($\log_{10}(\text{TCID}_{50})\ g^{-1}$)[a]	sheep no. measurements
extremity lesion	[40]	7.4	2	[35]	9.5	2	[41]	8.4	5
	AVE	7.4		[42]	9.0	2	AVE	8.4	
				[43]	8.0	16			
				AVE	8.8				
extremity no lesion	[39]	6.4	11	[35]	6.0	4			
	[8]	4.0	21	[42]	6.5	n.a.[b]			
	[40]	4.9	4	AVE	6.3				
	AVE	5.1					AVE	n.a.	
non-extremity	[39]	5.0	6	[43]	4.3	6			
	[8]	4.0	54	AVE	4.3				
	AVE	4.5					AVE	n.a.	

[a] Units reported are TCID$_{50}$—the amount of virus required to infect 50% of calf thyroid tissue (BTY) cultures [44]. Measurements reported using methods other than BTY cultures have been scaled. Measurements reported below the instrument detection limits are assumed to be 0 for calculation purposes. See electronic supplementary material, table S1, for details.
[b] Scaled from lesion results based on data reported in Monaghan et al. [42].

section. This estimate does not assume that all shed skin cells contain the same amount of infectious FMDV.

For perspective, it is informative to note that a recent review of the FMD infectious dose via the aerosol route suggested that the minimum FMD infectious dose is 11 TCID$_{50}$ for sheep, 25 TCID$_{50}$ for cattle and 180 TCID$_{50}$ for swine [47]. The estimated peak FMDV skin cell emission rate of approximately 10^6 TCID$_{50}$ per animal per day for swine and cattle exceeds these figures by orders of magnitude, and so, in theory, FMDV could be transmitted via an infected skin cell pathway.[9] This daily FMD excretion rate from exfoliated skin cells is approximately the same magnitude as that estimated to be due to urine or faeces [1]. It is also about 10 to 100 times greater than the FMD aerosol emissions measured directly from infected swine respiratory systems [5]. There are, however, important unknowns in the latter comparison. For example, the latter study did not account for aerosol losses and so probably underestimated the total respiratory emissions.

3. PROVIDING CONTEXT TO THE HYPOTHESIZED FOOT AND MOUTH DISEASE SKIN AEROSOL SOURCE

(a) *Timing of foot and mouth disease virus aerosol emissions*

The timing of FMDV emergence in skin tissue is consistent with the skin being a source of infectious aerosols. In swine (but less clearly in cattle and sheep), emissions of airborne virus are observed to begin (and peak) coincident with the onset of clinical signs of FMD (e.g. the development of visible lesions outside the inoculation site)—the time when FMDV skin concentrations peak. Emissions then persist for several days [1,5,7,48,49]. While this may generally be the case, airborne FMD has occasionally been observed to begin on the day before clinical signs appear or alternatively to begin as much as several days after the development of clinically evident lesions. However, a general association of FMDV aerosol emissions with clinical skin lesion development is particularly strong in the swine experiments in which infection occurred via airborne or direct contact.[10] In these experiments, most animals emitted no airborne virus prior to skin lesion development and no airborne emissions were reported more than 1 day prior to the development of the clinical signs of FMD [5,7,50].

(b) *Whole-animal foot and mouth disease virus aerosol emission rates*

While FMD was first proved to be capable of airborne spread in the 1930s [51], it was not until the 1960s that detailed experiments were first performed to characterize the emission of infectious FMD aerosols. Many of the published laboratory studies of FMD aerosol emissions were performed at the UK Institute of Animal Health and have been performed using similar experimental conditions. While it is beyond the scope of this study to provide a detailed review of the kinetics and magnitude of FMD aerosol emissions, table 2 and electronic supplementary material, table S2 provide a summary of published estimates of the peak whole-animal FMD aerosol emission rate (i.e. the average emission rate per animal per 24 h period[11] for the day of maximum emissions).[12] The total amount of FMDV collected by the air sampler was converted into a 24 h emission rate using equation

Table 2. Peak whole-animal FMDV aerosol emission rates. AVE, average of values.

	cattle			swine			sheep		
	study	average peak FMDV aerosol emissions ($\log_{10}(\text{TCID}_{50})$/ animal/day)	no. measurements	study	average peak FMDV aerosol emissions ($\log_{10}(\text{TCID}_{50})$/ animal/day)	no. measurements	study	average peak FMDV aerosol emissions ($\log_{10}(\text{TCID}_{50})$/ animal/day)	no. measurements
	[52]	4.0	4	[53]	6.9	2	[52]	5.2	1
	[54]	4.1	9	[50]	7.6	1	[54]	2.7	8
	[7]	4.7	3	[7]	6.4	2	[55]	5.4	1
	[6]	4.4	3	[56]	7.3	8	[7]	4.1	2
				[54]	6.0	8			
				[49]	8.1	8			
				[48]	8.5	1			
				[6]	7.4	7			
	AVE	4.3		AVE	7.3		AVE	4.3	

(3.1)[13] and airborne FMDV concentrations either directly reported or calculated from equation (3.2). Equation (3.1) was derived assuming a steady-state air concentration (i.e. losses within the animal holding area are balanced by animal emissions), well-mixed air (i.e. air concentrations are the same at all locations within the loosebox) and a $4 \times 3 \times 3$ m (3.6×10^4 l) loosebox.

$$\text{FMDV}_{\text{emissions}} = \frac{[\text{FMDV}]_{\text{air}} \times V_{\text{loosebox}} \times (L_{\text{aerosol}} + L_{\text{ACH}})}{N_{\text{i}}},$$

$$(3.1)$$

where $\text{FMDV}_{\text{emissions}}$ is the FMDV aerosol emission rate in TCID_{50} per animal per day, $[\text{FMDV}]_{\text{air}}$ is the measured FMDV air concentration in TCID_{50} per litre, V_{loosebox} is the loosebox volume, L_{aerosol} is the measured loosebox FMDV aerosol loss rate with no air exchange (144 per day) (§3c), L_{ACH} is the air exchange rate during the sampling period and N_{i} is the number of infected (FMDV excreting) animals in the loosebox.

$$[\text{FMDV}]_{\text{air}} = \frac{\text{total FMDV collected}}{\text{air flow rate} \times t_{\text{sampling}}},$$

$$(3.2)$$

where total FMDV collected is the total amount of FMDV in the liquid sampling media in TCID_{50}, air flow rate is the sampling instrument air flow rate in litres per minute and t_{sampling} is the sampling duration in minutes.

Overall, the average per animal peak FMDV aerosol emission rate is estimated to be approximately 10^7 TCID_{50} per day for swine and $10^{4.5}$ TCID_{50} per day for cattle and sheep. These whole-animal emission values are similar in magnitude to the infected skin cell shedding rate of 10^6 TCID_{50} per day previously estimated for swine and cattle. One study compared whole-animal (swine) infectious aerosol emission rates from live and dead animals, and reported that FMDV aerosol concentrations (and thus emission rates) decreased by 10–100-fold when animals were slaughtered [6]. The dead swine FMDV aerosol emission rate was similar to that reported above for (live) sheep and cattle, and is 10 per cent of the total infected FMD skin cell shed rate estimated in §2c.

(c) Foot and mouth disease virus stability in detached skin and whole-animal aerosols

While there are no studies examining the stability of FMDV in skin aerosols, there are a few studies that have examined FMDV stability in skin separated from live animals (i.e. skin not subject to *in vivo* antibody clearance). The available data suggest that the FMDV lifetime in detached skin is long—from days to months. Sellers *et al.* [6] demonstrated that FMDV concentrations in swine foot lesions did not decrease over a 24 h period. Gailiunas & Cottral [57] demonstrated that FMDV in clinically normal bovine hides consistently remained infectious (and virulent) for weeks to months in storage. These samples were either dried (20°C, 40% humidity) or salt/brine-cured (temperatures ranged from 4°C to 15°C and humidity ranged from 40 to 90%).

The two related studies that examined *in situ* FMDV aerosol stability of naturally generated aerosols suggest that the lifetime of naturally generated aerosols is similarly long. Sellers *et al.* [6] and Sellers & Herniman [58] examined the quantity of airborne FMDV in animal-holding pens (looseboxes) both prior to and after killing infected

swine and cattle.[14] Only the swine measurements are discussed in detail here as these experiments were more extensive and the FMDV signal was higher (the results for cattle also suggest a long aerosol lifetime). FMDV aerosol emissions were measured under four experimental conditions: (i) in boxes in which live swine were held, (ii) in boxes in which live swine were placed and then removed (without being killed), (iii) in boxes in which live swine were placed and then killed (bodies remained in the box), and (iv) in clean boxes in which freshly killed swine bodies were placed. Overall (non-size-resolved) airborne FMDV concentrations in swine-holding pens were observed to decrease by 10–1000-fold at 30 min and 24 h, respectively, after live animals were removed (see electronic supplementary material, table S2 for more details). Separate measurements over a 1 h time period suggest that most of the decrease in airborne infectivity was associated with large (greater than 6 µm) aerosols and that for small (less than 3 µm) aerosols infectivity decreased less than 10-fold over a 1 h time period. Gravitational settling of suspended aerosols could explain such loss rates[15]—indicating a limited loss rate (much less than 10-fold in 1 h) of FMDV infectivity in airborne aerosols.

It is important to note that the aerosol stability estimates provided by these experiments do not provide any insight into the relative importance of the skin versus respiratory emission sources. The experiments reported by Sellers *et al.* [6] and Sellers & Herniman [58] were performed at high (greater than 90%) relative humidity. Laboratory experiments on synthetic aerosols generated from liquid FMDV suspensions have reported high-humidity aerosol decay rates that range from near zero to 1000-fold per hour, depending on the virus strain and the suspending fluid used [59–62].

(d) Aerosol size
The size fractionation typically reported for fresh FMD aerosol emissions is 10 to 30 per cent in less than 3 µm, 20 to 40 per cent in 3–6 µm and 30 to 70 per cent in greater than 6 µm aerosols [5,7,48,58]. These measurements have been made in swine, and no aerosol size fractionization distribution data appear to be available for cattle or sheep emissions. The measured size distribution for swine is consistent with what is known for mammalian skin cell aerosols—which are emitted in a variety of aerosol sizes but on average are large (approximately 14 µm; §2a). In addition, if the measured loosebox aerosol loss rates derived from the Sellers *et al.* [6] and Sellers & Herniman [58] data (electronic supplementary material, table S2) are assumed to be due solely to gravitational settling, then the corresponding effective aerosol settling velocity of 0.3 m min^{-1} agrees well with that found for skin aerosols [10,18].

4. DISCUSSION
(a) Recommendations for additional experiments
The literature summarized above provides considerable evidence for the hypothesis that animal skin cells could be a significant source of infectious FMDV aerosols. However, there are important knowledge gaps. Studies are outlined below that could significantly contribute to affirming or disproving this hypothesis.

First, the FMDV concentration in the outermost skin layer that normally exfoliates (stratum corneum) needs to be characterized. This could potentially be accomplished by analysing skin samples from the bodies of infected animals using a skin surface sampling technique such as skin scraping (with care to select only the top layer of the epidermis) or skin scrubbing [63]. Follow-on work, if warranted, could characterize (i) the infectivity and stability of FMDV in these skin cells, (ii) the degree to which infectious FMD in exfoliated skin cells is intracellular versus viral rafting on the surface, (iii) the emissions rate of airborne infectious FMD skin cells, (iv) the infectious aerosols collected during whole-animal sampling and (v) the infectivity of environmentally aged (e.g. dust mite-processed) skin aerosols.

Second, the Sellers *et al.* [6] and Sellers & Herniman [58] experiments should be repeated. These studies are unique (and therefore should be verified) because they are the only experiments identified that have examined (i) the FMDV aerosol emission rate from dead animals, (ii) the relative importance of respiratory versus non-respiratory emission pathways (suggested from the results of whole-animal FMDV aerosol emissions from live and dead animals) and (iii) the time series of aerosol concentrations from whole animals when animals were removed from the measurement chamber (these data were used to infer the stability of infectious FMDV in natural aerosols). Key extensions to this work include the use of domestic animals other than swine and testing in environments with lower relative humidity.

(b) Implications for foot and mouth disease control
If further testing were to support the study hypothesis, then there are a number of practical implications for FMD surveillance and control.

First, the sampling and management of settled dust could prove to be a useful tool for disease surveillance and control. Owing to (i) the potentially high stability of FMDV in skin and (ii) the high fraction of exfoliated skin fragments in settled dust, FMDV could remain detectable (and indeed potentially infectious) in dust for months or years after a primary infection. The re-aerosolization of FMDV-infected settled dust could therefore prove to be a significant concern (electronic supplementary material, Particle Suspension Mechanisms).

Second, slaughtered animals may still emit airborne FMDV via continued exfoliation of infected skin cells simply by exposure to air currents (e.g. wind) and/or external mechanical abrasion (e.g. moving animal carcasses, spraying hides with water).

Third, the current focus on swine airborne emissions (and the relative neglect of cattle and sheep emissions) may need to be revisited. It is well known that hair can trap aerosols. Of the three animals considered, pigs are known to be the highest FMD aerosol emitters and also have the lowest body hair count. Therefore, while sheep (and to a lesser extent cattle) may typically have limited ability to shed skin aerosols through their coat into the atmosphere, shearing or similar actions that disturb the coat and/or skin could theoretically release infectious FMDV aerosols well after the obvious acute clinical infection has been cleared from the animal.

(c) Implications for other diseases
If further work supports the study hypothesis with respect to FMDV, the role of skin cell aerosols in spreading other viral diseases may need to be revisited (electronic supplementary material, Other Viral Diseases). Viral disease

Table 3. Key study findings.

key finding	level of certainty	new data needed
key findings from prior studies		
FMDV is trophic for animal skin	well established	
skin is a major secondary FMD viral replication site	well established	
FMDV is present both in skin lesions and in skin appearing clinically normal	probable	
FMDV skin concentrations are highest in the epidermal layer	probable	FMDV concentration and infectivity of apparently normal stratum corneum samples (by species and body region)
in the normal skin growth cycle, epidermal skin cells are shed into the environment	well established	
skin cells constitute a significant fraction of ambient aerosols and settled dust	well established	
skin cell aerosols can deposit within the respiratory system	probable	
airborne skin cells are a known vehicle for disease transmission	well established for bacteria; probable for viruses (e.g. VZV)	measurement of concentration and infectivity of FMDV on exfoliated skin cell surface and intracellularly in fresh and environmentally aged skin cells
dead animals emit infectious aerosols	probable	confirmatory studies; current data come from a single study
peak FMDV aerosol emissions are coincident with peak FMDV skin concentrations	well established	
FMDV has high stability in detached (whole animal) skin	probable	confirmatory studies; current data come from two studies
key findings from this study		
estimates of the peak FMDV-infected animal skin cell shedding rate		
— are comparable to measured peak whole-animal aerosol emissions	probable	skin cell shedding rates for domestic animals; updated FMDV skin concentrations
— exceed the minimum infectious dose by orders of magnitude	possible	degree to which FMDV is liberated from skin cells in the respiratory system
stability of naturally generated infectious FMDV aerosols is consistent with that expected of FMDV-infected skin aerosols	possible	confirmatory studies; conclusion based on data from a single study and assumption that FMDV stability in skin aerosols is comparable to whole skin
the whole-animal FMDV infectious aerosol size distribution is consistent with that expected for skin cell aerosols	well established	enhanced characterization of (i) skin aerosol size distribution and (ii) infectious whole-animal FMDV aerosol size distribution
utility of study hypothesis		
may point to new methods for FMD surveillance (e.g. settled dust)	possible	stability and infectivity of FMDV in dust
potential to develop new, more effective disease control measures	possible	degree to which infectious skin cells contribute to disease transmission
may lead to new studies on the persistence of the virus in the environment	possible	analysis of settled dust and other potential environmental reservoirs
may lead to better understanding of sources and vehicles of infectious aerosols with applicability to other diseases	possible	degree to which infectious skin cells contribute to viral disease transmission

spread via skin cell aerosol is given minimal treatment or is entirely absent in recent literature reviews [64–66]. Given the potential for skin cells to provide protection to infectious virus against adverse environmental conditions, the management of several viral diseases may also benefit from enhanced dust surveillance and management, and skin decontamination.

5. SUMMARY AND CONCLUSIONS
There is considerable evidence in the literature to support the hypothesis that infected animal skin cells could be a significant source of infectious FMDV aerosols. Table 3 provides a summary of both key findings and suggested future research.

ENDNOTES
[1] Other potential sources of infectious FMDV aerosols were not ruled out by this study, nor by an earlier study [67] that reported more virus recovered from the noses of animal handlers examining the head relative to other handlers examining other body regions.

[2] Measurements reported here were taken near human habitats. Skin cells may not contribute significantly to the total atmospheric aerosol burden at locations well removed from human/animal habitation (e.g. remote ocean).

[3] Aerodynamic diameter is a measure of how the aerosol will behave in the atmosphere and does not necessarily equal the physical aerosol dimension(s). This study uniformly uses this metric to compare aerosols.

[4] Epidermal thickness is known to vary between the glabrous (e.g. snout) and haired regions with a lesser variation between animal species [68]. The value chosen here is more reflective of the haired regions, where published epidermal thicknesses include 60 μm in

cattle [33], 30–100 μm and 70–140 μm in swine [30], and 50 μm in sheep [34]. The nominal value used in this study includes both the living and non-living portions of the epidermis. This value was chosen to allow direct comparison with skin/epidermis FMD concentration measurements (data on FMD concentrations in the stratum corneum are not available).

[5] Emission rates are scaled from human emission rates based on relative surface area. Surface areas of 0.7 m^2 (swine), 2.9 m^2 (cattle) and 0.8 m^2 (sheep) were calculated assuming a 30 kg swine, 200 kg cow and 30 kg sheep using the methods described by Kelly et al. [69] and Berman [70]. Animal sizes were chosen to reflect animals used in FMD aerosol emission studies. For context, the adult human body surface area is 1.75 m^2 [28].

[6] Peak skin concentrations are typically coincident (or at most within a single 24 h sampling period) of the development of widespread visible (macroscopic) lesions, typically a few days after the initial infection [35,37,39,40]. FMDV levels in live animal skin tissues significantly decrease after antibodies begin to circulate a few days later. FMDV RNA (but not infectious FMD) has been reported in skin up to several weeks after infection [1,40,43,71].

[7] Presumably external contamination of the skin could also occur with other FMD-laden excretions. As summarized by Alexandersen et al. [1], many body excretions, such as oral saliva, nasal secretions, urine and faeces, contain infectious FMDV.

[8] There are insufficient data on sheep skin concentrations to justify an emissions estimate.

[9] There are no data on the degree to which infectious FMDV could be released from the airborne skin cells that deposit within the respiratory system.

[10] Other infection routes (e.g. inoculation in a foot) and the high-dose exposure regimen typically used to accelerate the rate of disease progression often yielded clinically evident lesions in the first 24 h (smaller than the sampling timescale).

[11] The reported values are normalized. The sampling period ranged from 5 min to 1 h.

[12] The data reported correspond to loosebox experiments performed at UK Institute of Animal Health and assume similar aerosol loss rates. Additional data are available for a small (610 l) sampling chamber. However, aerosol loss rates in this chamber have not been reported in the published literature and so equations (3.1) or (3.2) cannot be used.

[13] This equation differs from that previously used in the literature [49], but incorporates new effects such as the FMDV aerosol loss rate and the size of the loosebox. The values reported here are broadly consistent with, although higher than, those previously reported.

[14] In Sellers et al. [6], sampling took place after the generalization of FMD. Lesion epithelium taken from swine feet during this experiment correspond to 10^9 TCID$_{50}$ per gram of tissue. In Sellers & Herniman [58], sampling took place 48 and 72 h after inoculation and when generalized lesions were evident. Humidity was kept above 90 per cent.

[15] Assuming the air within the 3 m high loosebox is well-mixed, gravitational settling would remove 30 per cent of the 3 μm aerosols and 70 per cent of the 6 μm aerosols in the first hour. After 24 h, only 10^{-4} and 10^{-13} of the 3 and 6 μm original aerosol mass, respectively, would be expected to remain airborne.

REFERENCES
1 Alexandersen, S., Zhang, Z., Donaldson, A. I. & Garland, A. J. M. 2003 The pathogenesis and diagnosis of foot-and-mouth disease. *J. Comp. Pathol.* **129**, 1–36. (doi:10.1016/S0021-9975(03)00041-0)

2 Smith, L. P. & Hugh-Jones, M. E. 1969 The weather factor in foot and mouth disease epidemics. *Nature* **223**, 712–715. (doi:10.1038/223712a0)

3 Christensen, L. S., Normann, P., Thykier-Nielsen, S., Sørensen, J. H., de Stricker, K. & Rosenørn, S. 2005 Analysis of the epidemiological dynamics during the 1982–1983 epidemic of foot-and-mouth disease in Denmark based on molecular high-resolution strain

identification. *J. Gen. Virol.* **86**, 2577–2584. (doi:10.1099/vir.0.80878-0)

4 Gloster, J., Champion, J., Sørensen, J. H., Mikkelsen, T., Ryall, D. B., Astrup, P., Alexandersen, S. & Donaldson, A. I. 2003 Airborne transmission of foot-and-mouth disease virus from Burnside Farm, Heddon-on-the-Wall, Northumberland, during the 2001 epidemic in the United Kingdom. *Vet. Rec.* **152**, 525–533. (doi:10.1136/vr.152.17.525)

5 Donaldson, A. I. & Ferris, N. P. 1980 Sites of release of airborne foot-and-mouth disease virus from infected pigs. *Res. Vet. Sci.* **29**, 315–319.

6 Sellers, R. F., Herniman, K. A. J. & Donaldson, A. I. 1971 The effects of killing or removal of animals affected with foot-and-mouth disease on the amounts of airborne virus present in looseboxes. *Br. Vet. J.* **127**, 358–365.

7 Sellers, R. F. & Parker, J. 1969 Airborne excretion of foot-and-mouth disease virus. *J. Hyg. Camb.* **67**, 671–677. (doi:10.1017/S0022172400042121)

8 Gailiunas, P. & Cottral, G. E. 1966 Presence and persistence of foot-and-mouth disease virus in bovine skin. *J. Bacteriol.* **91**, 2333–2338.

9 Alexandersen, S., Zhang, Z. & Donaldson, A. I. 2002 Aspects of the persistence of foot-and-mouth disease virus in animals—the carrier problem. *Microbe. Infect.* **4**, 1099–1110. (doi:10.1016/S1286-4579(02)01634-9)

10 Clark, R. P. & de Calcina-Goff, M. L. 2009 Some aspects of the airborne transmission of infection. *J. R. Soc. Interface* **6**, S767–S782. (doi:10.1098/rsif.2009.0236.focus)

11 Wickett, R. R. & Visscher, M. O. 2006 Structure and function of the epidermal barrier. *Am. J. Infect. Control* **34**, S98–S110. (doi:10.1016/j.ajic.2006.05.295)

12 Milstone, L. M. 2004 Epidermal desquamation. *J. Dermatol. Sci.* **36**, 131–140. (doi:10.1016/j.jdermsci.2004.05.004)

13 Dawson, J. R. 1990 Minimizing dust in livestock buildings: possible alternatives to mechanical separation. *J. Agric. Eng. Res.* **47**, 235–248. (doi:10.1016/0021-8634(90)80044-U)

14 Herber, A. J., Stroik, M., Faubion, J. M. & Willard, L. H. 1988 Size distribution and identification of aerial dust particles in swine finishing buildings. *Trans. ASAE* **31**, 882–887.

15 Clark, R. P. 1974 Skin scales among airborne particles. *J. Hyg.* **72**, 47–51. (doi:10.1017/S0022172400023196)

16 Clark, R. P. & Shirley, S. G. 1973 Identification of skin in airborne particulate matter. *Nature* **246**, 39–40. (doi:10.1038/246039a0)

17 Noble, W. C. (ed.) 1993 *The skin microflora and microbial skin disease*. New York, NY: Cambridge University Press.

18 Hambraeus, A. 1988 Aerobiology in the operating room—a review. *J. Hosp. Infect.* **11**(Suppl. A), 68–76. (doi:10.1016/0195-6701(88)90169-7)

19 Noble, W. C., Habbema, J. D. F., van Furth, R., Smith, I. & deRaay, C. 1976 Quantitative studies on the dispersal of skin bacteria into the air. *J. Med. Microbiol.* **9**, 53–61. (doi:10.1099/00222615-9-1-53)

20 Davies, R. R. & Noble, W. C. 1962 Dispersal of bacteria on desquamated skin. *Lancet* **280**, 1295–1297. (doi:10.1016/S0140-6736(62)90849-8)

21 Davies, R. R. & Noble, W. C. 1963 Dispersal of staphylococci on desquamated skin. *Lancet* **281**, 1111. (doi:10.1016/S0140-6736(63)92159-7)

22 Duguid, J. P. & Wallace, A. T. 1948 Air infection with dust liberated from clothing. *Lancet* **2**, 845–849. (doi:10.1016/S0140-6736(48)91428-7)

23 Lewis, H. E., Foster, A. R., Mullan, B. J., Cox, R. N. & Clark, R. P. 1969 Aerodynamics of human microenvironment. *Lancet* **293**, 1273–1277. (doi:10.1016/S0140-6736(69)92220-X)

24 Clark, R. P. & Cox, R. N. 1973 The generation of aerosols from the human body. In *Airborne transmission and airborne infection* (eds J. F. P. Hers & K. C. Winkler), pp. 413–426. Utrecht, The Netherlands: Oosthoek Publishing Co.

25 MacKintosh, C. A., Lidwell, O. M., Towers, A. G. & Marples, R. R. 1978 The dimensions of skin fragments dispersed into the air during activity. *J. Hyg. Camb.* **81**, 471–479. (doi:10.1017/S0022172400025341)

26 May, K. R. & Pomeroy, N. P. 1973 Bacterial dispersion from the body surface. In *Airborne transmission and airborne infection* (eds J. F. P. Hers & K. C. Winkler), pp. 428–432. Utrecht, The Netherlands: Oosthoek Publishing Co.

27 Cleton, F. J., Van der Mark, Y. S. & van Toorn, M. J. 1968 Effect of shower-bathing on dispersal of recently acquired transient skin flora. *Lancet* **291**, 865. (doi:10.1016/S0140-6736(68)90334-6)

28 Noble, W. C. 1975 Dispersal of skin microorganisms. *Br. J. Dermatol.* **93**, 477–485. (doi:10.1111/j.1365-2133.1975.tb06527.x)

29 Mortensen, J. T., Brinck, P. & Lichtenberg, J. 1998 The minipig in dermal toxicology. A literature review. *Scan. J. Lab. Anim. Sci.* **25**, 77–83.

30 Vardaxis, N. J., Brans, T. A., Boon, M. E., Kreis, R. W. & Marres, L. M. 1997 Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. *J. Anat.* **190**, 601–611. (doi:10.1046/j.1469-7580.1997.19040601.x)

31 Kangesu, T., Navsaria, H. A., Manek, S., Shurey, C. B., Jones, C. R., Fryer, P. R., Leigh, I. M. & Green, C. J. 1993 A porcine model using skin graft chambers for studies on cultured keratinocytes. *Br. J. Plast. Surg.* **46**, 393–400. (doi:10.1016/0007-1226(93)90045-D)

32 Chapman, S. J. & Walsh, A. 1990 Desmosomes, corneosomes and desquamation. An ultrastructural study of adult pig epidermis. *Arch. Dermolog. Res.* **282**, 304–310. (doi:10.1007/BF00375724)

33 Lloyd, D. H., Dick, W. D. B. & Jenkinson, D. M. 1979 Structure of the epidermis in Ayrshire bullocks. *Res. Vet. Sci.* **26**, 172–179.

34 Lloyd, D. H., Amakiri, S. F. & Jenkinson, D. M. 1979 Structure of the sheep epidermis. *Res. Vet. Sci.* **26**, 180–182.

35 Alexandersen, S., Oleksiewicz, M. B. & 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. *J. Gen. Virol.* **82**, 747–755.

36 Oleksiewicz, M. B., Donaldson, A. I. & Alexandersen, S. 2001 Development of a novel real-time RT–PCR assay for quantitation of foot-and-mouth disease virus in diverse porcine tissues. *J. Virol. Methods* **92**, 23–35. (doi:10.1016/S0166-0934(00)00265-2)

37 Brown, C. C., Olander, H. J. & Meyer, R. F. 1995 Pathogenesis of foot-and-mouth disease in swine studied by in-situ hybridization. *J. Comp. Pathol.* **113**, 51–58. (doi:10.1016/S0021-9975(05)80068-4)

38 Cottral, G. E. 1969 Persistence of foot-and-mouth disease virus in animals, their products and the environment. *Bull. Off. Int. Epiz.* **71**, 549–568.

39 Gailiunas, P. 1968 Microscopic skin lesions in cattle with foot-and-mouth disease. *Arch. für die gesamte Virusforsch.* **25**, 188–200. (doi:10.1007/BF01258164)

40 Zhang, Z. & Alexandersen, S. 2004 Quantitative analysis of foot-and-mouth disease virus RNA loads in bovine tissues: implications for the site of viral persistence. *J. Gen. Virol.* **85**, 2467–2575. (doi:10.1099/vir.0.80011-0)

41 Ryan, E., Horsington, J., Durand, S., Brooks, H., Alexandersen, S., Brownlie, J. & Zhang, Z. 2008 Foot-and-mouth disease virus infection in young lambs:

pathogenesis and tissue trophism. *Vet. Microbiol.* **127**, 258–274. (doi:10.1016/j.vetmic.2007.08.029)

42 Monaghan, P., Simpson, J., Murphy, C., Durand, S., Quan, M. & Alexandersen, S. 2005 Use of confocal immunofluorescence microscopy to localize viral non-structural proteins and potential sites of replication in pigs experimentally infected with foot-and-mouth disease virus. *J. Virol.* **79**, 6410–6418. (doi:10.1128/JVI.79.10.6410-6418.2005)

43 Lee, S. H., Jong, M. H., Huang, T. S., Lin, Y. L., Wong, M. L., Liu, C. I. & Chang, T. J. 2009 Pathology and viral distributions of the porcinophilic foot and mouth disease virus strain (O/Taiwan/97) in experimentally infected pigs. *Transbound. Emerg. Dis.* **56**, 189–201. (doi:10.1111/j.1865-1682.2009.01079.x)

44 Snowdon, W. A. 1966 Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature* **210**, 1079–1080. (doi:10.1038/2101079a0)

45 Durand, S., Murphy, C., Zhang, Z. & Alexandersen, S. 2008 Epithelial distribution and replication of foot-and-mouth disease virus RNA in infected pigs. *J. Comp. Path.* **139**, 86–96. (doi:10.1016/j.jcpa.2008.05.004)

46 Brown, C. C., Meyer, R. F., Olander, H. J., House, C. & Mebus, C. A. 1992 A pathogenesis study of foot-and-mouth disease in cattle using *in situ* hybridization. *Can. J. Vet. Res.* **56**, 189–193.

47 Sellers, R. & Gloster, J. 2008 Foot-and-mouth disease: a review of intranasal infection of cattle, sheep and pigs. *Vet. J.* **177**, 159–168. (doi:10.1016/j.tvjl.2007.03.009)

48 Gloster, J., Williams, P., Doel, C., Esteves, I., Coe, H. & Valarcher, J. F. 2007 Foot-and-mouth disease—quantification and size distribution of airborne particles emitted by healthy and infected pigs. *Vet. J.* **174**, 42–53. (doi:10.1016/j.tvjl.2006.05.020)

49 Gloster, J., Doel, C., Gubbins, S. & Paton, D. J. 2008 Foot-and-mouth disease: measurements of aerosol emission from pigs as a function of virus strain and initial dose. *Vet. J.* **177**, 374–380. (doi:10.1016/j.tvjl.2007.06.014)

50 Alexandersen, S., Quan, M., Murphy, C., Knight, J. & Zhang, Z. 2003 Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J. Comp. Pathol.* **129**, 268–282. (doi:10.1016/S0021-9975(03)00045-8)

51 Donaldson, A. I. 1983 Quantitative data on airborne foot-and-mouth disease virus: its production, carriage and deposition. *Phil. Trans. R. Soc. Lond. B* **302**, 529–534. (doi:10.1098/rstb.1983.0072)

52 Alexandersen, S., Zhang, Z., Reid, S. M., Hutchings, H. & Donaldson, A. I. 2002 Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. *J. Gen. Virol.* **83**, 1915–1923.

53 Alexandersen, S. & Donaldson, A. I. 2002 Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. *Epidemiol. Infect.* **128**, 313–323. (doi:10.1017/S0950268801006501)

54 Donaldson, A. I., Herniman, K. A. J., Parker, J. & Sellers, R. F. 1970 Further investigations on the airborne excretion of foot-and-mouth disease virus. *J. Hyg. Camb.* **68**, 557–564. (doi:10.1017/S0022172400042480)

55 Esteves, I., Gloster, J., Ryan, E., Durand, S. & Alexandersen, S. 2004. Appendix 35—Natural aerosol transmission of foot and mouth disease in sheep. See http://www.fao.org/ag/againfo/commissions/docs/research_group/greece04/App35.pdf.

56 Donaldson, A. I., Ferris, N. P. & Gloster, J. 1982 Air sampling of pigs infected with foot-and-mouth disease virus: comparison of litton and cyclone samplers. *Res. Vet. Sci.* **33**, 384–385.

57 Gailiunas, P. & Cottral, G. E. 1967 Survival of foot-and-mouth disease virus in bovine hides. *Am. J. Vet. Res.* **28**, 1047–1053.

58 Sellers, R. F. & Herniman, K. A. J. 1972 The effects of spraying on the amounts of airborne foot-and-mouth disease virus present in loose-boxes. *J. Hyg.* **70**, 551–556. (doi:10.1017/S0022172400063130)

59 Barlow, D. F. & Donaldson, A. I. 1973 Comparison of the aerosol stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. *J. Gen. Virol.* **20**, 311–318. (doi:10.1099/0022-1317-20-3-311)

60 Donaldson, A. I. 1973 The influence of relative humidity on the stability of foot-and-mouth disease virus in aerosols from milk and faecal slurry. *Res. Vet. Sci.* **15**, 96–101.

61 Barlow, D. F. 1972 The aerosol stability of a strain of foot-and-mouth disease virus and the effects on stability of precipitation with ammonium sulphate, methanol or polyethylene glycol. *J. Gen. Virol.* **15**, 17–24. (doi:10.1099/0022-1317-15-1-17)

62 Donaldson, A. I. 1972 The influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. *J. Gen. Virol.* **15**, 25–33. (doi:10.1099/0022-1317-15-1-25)

63 Roberts, D. & Marks, R. 1980 The determination of regional and age variations in the rate of desquamation: a comparison of four techniques. *J. Invest. Dermatol.* **74**, 13–16. (doi:10.1111/1523-1747.ep12514568)

64 Barker, J., Stevens, D. & Bloomfield, S. F. 2001 Spread and prevention of some common viral infections in community facilities and domestic homes. *J. Appl. Microbiol.* **91**, 7–21. (doi:10.1046/j.1365-2672.2001.01364.x)

65 Eames, I., Tang, J. W., Li, Y. & Wilson, P. 2009 Airborne transmission of disease in hospitals. *J. R. Soc. Interface* **6**, S697–S702. (doi:10.1098/rsif.2009.0407.focus)

66 Tang, J. W., Li, Y., Eames, I., Chan, P. K. S. & Ridgway, G. L. 2006 Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises. *J. Hosp. Infect.* **64**, 100–114. (doi:10.1016/j.jhin.2006.05.022)

67 Sellers, R. F., Donaldson, A. I. & Herniman, K. A. J. 1970 Inhalation, persistence and dispersal of foot-and-mouth disease virus by man. *J. Hyg. Camb.* **68**, 565–573. (doi:10.1017/S0022172400042492)

68 Jenkinson, D. M. 1993 The basis of the skin surface ecosystem. In *The skin microflora and microbial skin disease* (ed. W. C. Noble), pp. 291–314. New York, NY: Cambridge University Press.

69 Kelly, K. W., Curtis, S. E., Marzan, G. T., Karara, H. M. & Anderson, C. R. 1973 Body surface area of female swine. *J. Anim. Sci.* **36**, 927–930.

70 Berman, A. 2003 Effects of body surface area estimates on predicted energy requirements and heat stress. *J. Dairy Sci.* **86**, 3605–3610. (doi:10.3168/jds.S0022-0302(03)73966-6)

71 Zhang, Z. & Bashiruddin, J. B. 2009 Quantitative analysis of foot-and-mouth disease virus RNA duration in tissues of experimentally infected pigs. *Vet. J.* **180**, 130–132. (doi:10.1016/j.tvjl.2007.11.010)