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# Understanding the transmission of foot-and-mouth disease virus at different scales

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Foot-and-mouth disease (FMD) is highly infectious, but despite the large quantities of FMD virus released into the environment and the extreme susceptibility of host species to infection, transmission is not always predictable. Whereas virus spread in endemic settings is characterised by frequent direct and indirect animal contacts, incursions into FMD-free countries may be seeded by low-probability events such as fomite or wind-borne aerosol routes. There remains a void between data generated from small-scale experimental studies and our ability to reliably reconstruct transmission routes at different scales between farms, countries and regions. This review outlines recent transmission studies in susceptible host species, and considers new approaches that integrate virus genomics and epidemiological data to recreate and understand the spread of FMD.

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## Characteristics of foot-and-mouth disease

Foot-and mouth disease (FMD) affects cloven-hoofed animals (including cattle, sheep, goats and pigs), and is caused by an RNA virus (FMDV) in the family Picornaviridae. Characteristically, vesicles develop, especially in epithelia around the mouth, feet and mammary glands. Case-fatality is usually low except in young stock, but productivity losses and costs associated with control can be substantial [1]. The disease is highly contagious, and the potential for infection of different domesticated and wildlife hosts, not all of which show obvious signs of disease, is a further challenge to control [2]. FMDV exists as seven discrete serotypes, and the disease mainly occurs in Africa and Asia, with global distribution mirroring poverty and livestock density [3•]. New virus strains evolve and emerge regularly and give rise to successive waves of infection, which sometimes spill over into FMD-free regions. Vaccination with killed vaccines is used on a large scale but the immunity induced is short lived and is serotype and sometimes strain specific [4•].

During acute infection, transmission is facilitated by virus shedding from ruptured vesicles and in bodily excretions and secretions, including breath, milk and semen [5] (Figure 1). Susceptible ruminants can be infected by very low doses of inhaled virus through direct contact with the breath of other acutely infected animals, or indirectly by resuspension of aerosols from contaminated materials. Pigs are relatively resistant to FMDV infection via inhalation routes [5]. Other routes of infection such as ingestion or through abrasions require a higher dose of virus. Depending on conditions, FMDV can survive for days to months in the environment and in various animal products including meat [6]. There is a rapid immune response to infection associated with FMDV clearance, but some ruminant hosts continue to harbour virus, becoming carriers with low and declining levels of FMDV in specific nasopharyngeal epithelial sites [7] and associated lymphoid tissues [8].

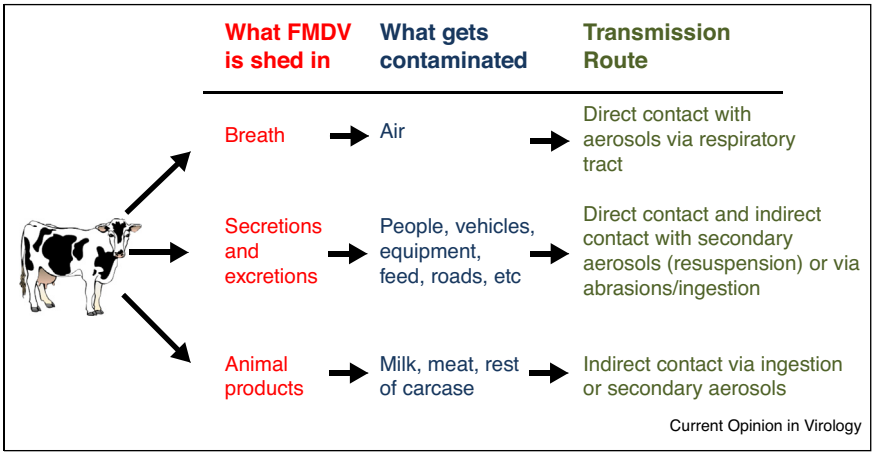
In the absence of obvious epidemiological links between infected animals, FMDV incursions into FMD-free countries must often be explained by low-probability events. This gives rise to the reputation of FMD as one of the most infectious diseases. A classic example was the long distance wind-borne spread of FMD to the Isle of Wight in the South of England in 1981 from a pig farm on the North French coast [9]. This contrasts with disease circulation within epidemics, or in and between countries where FMD is endemic, where spread occurs most readily via more predictable routes due to direct contact between animals and via traded animal products.

A challenge is to understand, quantify and model the multiplicity of different transmission routes possible for FMD at different scales in order to predict the disease's spread and the likely impact of control measures.

## Experimental studies of transmission

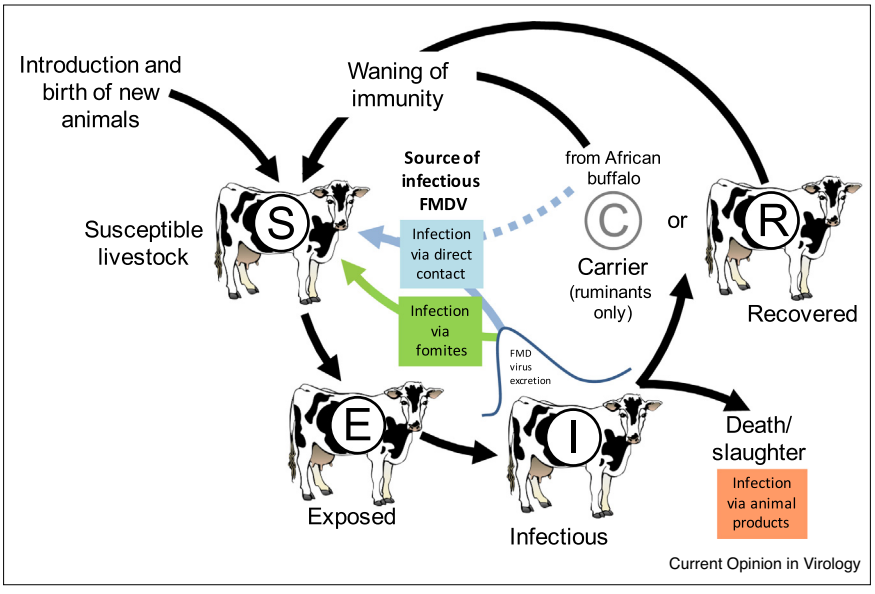
Experimental studies under controlled conditions have contributed enormously to our understanding of the pathogenesis and transmission dynamics of FMD (Figure 2), including sites of virus replication and persistence, incubation and shedding periods, minimum infectious doses by various routes, the nature and impact of the

**Figure 1**




Principal routes by which infectious FMD virus can be spread between susceptible animals (reviewed in [5]).

**Figure 2**




A simple S (susceptible), E (exposed) I (infected) and R (recovered) model describing cycles of FMDV replication and transmission in livestock. Susceptible animals can be infected via direct contact with infectious animals, through ingestion of infected animal products, via exposure to inanimate objects contaminated with FMDV (fomites), or through ingestion/aerosol contact with infected animal products. The period of infectiousness broadly correlates with the expression of clinical signs, although precise timing of these events has been observed to vary in experimental studies with different host species, infection models and FMDV serotypes.

immune response and differences between host species [5,10,11,12•]. It is important to recognise that these studies predominately focus on experimental infection in cattle, and consequently, transmission studies for other domesticated hosts (pigs, small ruminants and Asian buffalo) are under-represented in the literature. Furthermore, controlled studies with dangerous pathogens in animals are constrained by ethical, biosecurity, capacity and cost considerations. Small-scale studies lack the power to quantify low probability transmission routes, such as from fomites, contaminated feed or carriers. Thus, it is often difficult to quantify the force of infection arising from different transmission opportunities that may occur in the field and hence to recognise those of most importance under different circumstances.

A common difficulty for experimental studies is reconciling the need to design challenge models that reflect real-life

situations whilst remaining reproducible, comparable and quantifiable. In order to better mimic natural exposure, recent studies have pioneered the use of novel intranasopharyngeal (INP) inoculation systems in cattle [13], pigs [14], and sheep [15]. In cattle, these routes of infection were compared to direct contact exposure (cattle-to-cattle or pig-to-cattle) and to the conventionally used system of intraepithelial-lingual injection. There was more within-group variation in the timing of clinical infection following natural and simulated natural virus exposure systems when compared with needle inoculation. However, as well as more closely simulating field conditions, these alternative methods engage mucosal host defence mechanisms.

Most transmission experiments involve inoculating animals with FMDV and allowing them to mix with uninfected animals in a controlled environment. Novel experimental designs have been recently implemented in which the uninfected animals mix with the infected animals for defined periods of time, thus allowing FMDV infectiousness to be studied in more detail [16,17]. Recently, Stenfeldt and others studied direct contact transmission of FMDV within groups of pigs co-mingled in successive periods from 8 to 64 hours after inoculation of the donor pigs [17]. Infectiousness started 24 hours after inoculation but approximately 24 hours before the first clinical signs of FMD had appeared, coinciding with the start of viraemia in the donors. Furthermore, the onset and progression of clinical FMD in recipient pigs was faster after exposure to donors at more advanced phases of disease, suggesting this had resulted in a higher challenge dose. The shedding of FMDV in oropharyngeal fluids was a more reliable indicator of FMDV infectiousness than clinical signs. Importantly, these findings for groups of pigs differ from one-to-one transmission studies in cattle where animals were not infectious until, on average 0.5 days after clinical signs appeared [16], suggesting preclinical transmission is more likely from pigs than cattle. These examples highlight the challenges to establish generic insights that inform risk-based control policies using data from different host species, study designs, FMDV serotypes and infection methods.

Indirect transmission routes are notoriously difficult to quantify but are the main means for the spread of FMDV in countries that effectively impose movement bans in the face of disease outbreaks. An attempt to estimate indirect contact transmission rates in experimental calves has been recently reported [18]. This study estimated the reproduction ratio for transmission via the environment to be 2, which is at the level that would be able to sustain an epidemic. However, this estimate is substantially lower than the reproduction ratio reported for direct transmission, which is typically around 20–30 [19–21].

## Studying transmission in the field

In view of the limitations of experimental studies, field studies are essential, but have been hampered by a lack of capacity in parts of the world where FMD occurs, along with conflict between the requirements for expeditious disease control versus information gathering. Comprehensive information on both livestock epidemiology and disease progression is rarely available, but is needed to understand the relationship between animal production systems and the dynamics of FMD within them [22]. FMD control strategies are not always set up in a way that promotes their evaluation and few field trials of vaccination are undertaken [23]. Accurate modelling of field FMD transmission is further complicated by the contribution of sub-clinical infection amongst acutely infected animals, particularly between vaccinated animals where clinical disease is less evident [24–26].

A lack of detailed data also hampers the development of models to predict FMDV transmission and control, a topic reviewed by Pomeroy and others [27••]. They found that modelling methodology is well developed with multiple methods to represent contact-specific transmission and targeted control. However, detailed disease and host data representing large FMD outbreaks used to populate these models are restricted mainly to the UK epidemic of 2001. In fact, many insights have been gained from studying this epidemic, such as of the relatively greater contribution of cattle rather than sheep in disease transmission both between and within farms. But it is difficult to know how far these findings can be generalised to situations elsewhere, with different animals, husbandry systems, livestock and human networks, climates and levels of pre-existing immunity. Novel data sources are needed and notwithstanding the challenges involved, host and disease data should be collected from endemic settings to advance control and better understand FMDV transmission dynamics. As an example of the potential benefit of these approaches, a recent model of transmission from African buffalo to cattle adjacent to the Kruger National Park in South Africa [28••] reproduced the observed frequency of outbreaks, highlighting the importance of young buffalo and suggesting that cattle moving into the park may be more important than buffalo escaping.

## The role of carriers in transmission

The mechanisms involved in virus persistence within carrier ruminants and the very low risks for onward transmission are incompletely understood, hindering the development of risk-based approaches to inform disease control and international rules for safe trade in animals and their products [29]. Experimental and field studies to understand the epidemiological role of carrier animals continue to be a focus of research. Hayer and others monitored duration of the carrier state in Indian cattle and found the mean period of virus persistence was 13 months [30]. Cellular determinants of the carrier state have also been studied using host transcriptome analysis of tissue samples processed by laser capture microdissection from

experimentally infected cattle [7], indicating suppression of antiviral host factors in association with persistent FMDV. At the level of the host, Bronsvoort and others studied carriers within cattle herds in Cameroon, where FMD is endemic [31]. They confirmed that carrier rates decrease markedly with time after infection, and found that younger animals are more likely to be carriers. However, there was no evidence to support virus transmission from these carriers (via seroconversion of animals born after outbreaks).

African buffalo (*Syncerus caffer*), in which FMDV may have originated, can act as a reservoir host, maintaining the three Southern African serotypes (SAT 1-3) of FMDV, even within relatively small and isolated buffalo populations [32]. This probably involves transmission from carrier adults to susceptible young buffalo and carrier African buffalo have also been shown to transmit the virus to cattle. In contrast, transmission from carrier domestic livestock has not been proven by experimental studies [33]. Occasional spread of SAT serotypes to cattle can give rise to self-sustaining epidemics which are difficult to control with vaccination due to the antigenic diversity of the buffalo virus pools. Buffalo to cattle transmission is therefore another example of something that occurs rarely, but can be of great significance [21]. Coinfection studies in African buffalo identified infectious virus and viral genomes for up to 185 and 400 days respectively in lymphoid tissues of the head and neck, mainly in germinal centres [34]. There was a correlation between persistence and *in vitro* cell-killing capacity of different virus isolates, suggesting that the duration of persistence of FMDVs may be linked to their replication and cell-killing capacity.

## Transmission between regions

Spread of FMDV at higher scales (i.e. between farms, countries or regions) is a complex process affected by factors beyond virus shedding and uptake. For example, long distance airborne transmission of FMDV is dependent on specific virus transfer and survival conditions which can now be predicted by models that consider wind direction and strength, temperature, relative humidity and geographical topography [35]. Consequently, this mode of transmission is understood to be uncommon even in temperate zones and extremely unlikely in hot and arid conditions.

Factors associated with globalisation increasing the risk of long-distance spread of FMDV are well recognised, such as the movements of people, goods, animals and their products. Unrestricted and illegal movements are especially risky, whilst civil unrest disrupts disease and border controls and alters animal, people and trade flows [36,37]. There is a long history of using virus genetic data to study the international spread of FMDV [38–40], which continues with collaboration between reference laboratories supported by the Food and Agriculture Organisation of the United Nations and the World Animal Health Organisation (OIE) [41]. Recent studies show repeated and widespread dissemination of FMDV serotypes O and A from South Asia (Figure 3), which is perhaps counter-intuitive given the FMD control efforts and progress reported from India, the largest country in the region and the world's biggest cattle producer.

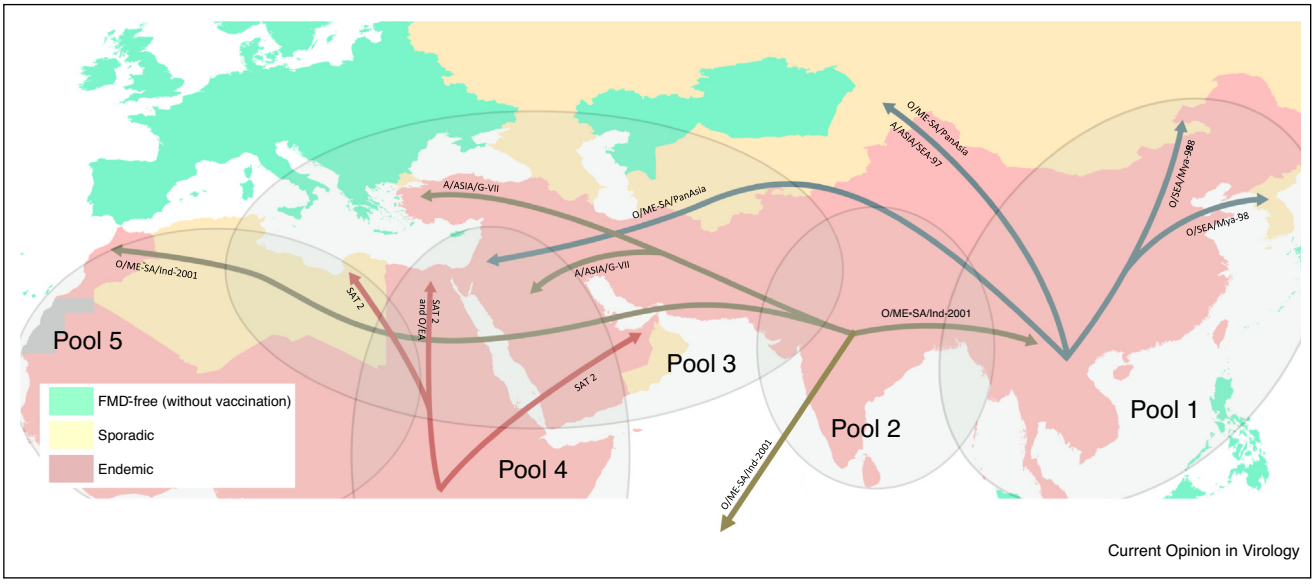
## Using phylodynamics to understand transmission

FMDV replicates and evolves rapidly allowing transmission and selection to be studied at different scales; between cells and tissues (i.e. within individual animals), between animals, between farms and between countries [42]. In their review, Pybus and Rambault discuss the emerging science of phylodynamics, which links pathogen evolution with the dynamics of infection and transmission [43•].

A primary aim of FMD researchers has been to use genetic data to resolve the order and timing of transmission events between outbreaks, since surveillance and epidemiological enquiries often miss infected farms and cannot resolve transmission pathways. Because of the many possible routes for virus spread, it is difficult to determine which has been responsible for new outbreaks. For example, the precise source and route of infection (who infected whom and how) for most of the >2000 premises infected during the 2001 UK epidemic could not be determined with certainty from epidemiological enquiries. Analysis of the overlapping periods within which individuals or groups are infected and/or infectious can help to decide the most likely direction of transmission, although this depends upon accurate observations on farms, combined with accurate estimates of incubation and shedding patterns. Many transmission permutations usually remain and constraining this selection with FMDV genetic data has been used to infer the most likely transmission routes between farms affected by FMD in the UK in 2001 and 2007 [44•,45]. Direct source attribution by sequencing can only inform the likelihood of direct linkage to the last 'replicating' source (an animal), a problem apparent in analysis of the earlier UK epidemic of 1967/8 [46].

The UK 2001 and 2007 datasets have been reanalysed with increasingly sophisticated methods [47–52]. These approaches attempt to overcome difficulties such as unobserved outbreaks on farms, incorrect attribution of infection and shedding times, unobserved evolution within unsampled hosts and lack of farm network and contact data. Although these methods provide information on who infected whom, including quantifying the uncertainty in attribution of transmission links, they cannot on their own tell us about the routes of transmission or their relative importance. However, these methods could

**Figure 3**




Recent long-distance spread of FMD viruses revealed by sequence analyses. Since 2008, a number of region-specific FMDV lineages that normally circulate and are maintained within endemic pools have spread into new geographical settings (coloured arrows represent viruses from sub-Saharan Africa (red), the Indian sub-continent (brown), and Southeast/East Asia (blue)). The southward pointing brown arrow denotes FMDV spread to cause an outbreak that occurred on Islands in the Indian Ocean during 2016 (Mauritius and Rodrigues). No single factor has been recognised that underpins these transboundary transmission patterns, which are probably exacerbated by the escalation of regional political crises, and migration of people in North Africa and the Middle East along with increased demand for animal products in East Asia.

play an immensely important role in understanding the epidemiology of FMDV in endemic settings, and particularly in the role of wildlife, and the management of livestock and wildlife interface, but this will require significant improvements in surveillance and sample acquisition.

More realistic models of within-host pathogen evolution would allow greater information about 'who-infected-whom' to be extracted from a phylogeny. Whole genome consensus sequencing of FMDVs obtained during serial passage in cattle confirmed the ability of phylogeny to reproduce the transmission events between animals [53]. These experimental studies were complemented by investigations of the variability of FMDV full genome consensus sequences from samples collected from animals representing multiple hosts on each of the holdings affected during the 2007 UK outbreak [54]. An average 4.6 nucleotide sequence differences accrued during transmission between infected farms, similar to the one to four nucleotide changes that were detected during controlled inter-animal transmissions. The small number of substitutions between farms suggests limited cycles of within-farm transmission prior to disease detection and sampling.

The advent of next generation sequencing (NGS) provides greater resolution to monitor the entire sequence swarm that exists within FMDV samples and the impact of transmission within and between hosts upon sub-consensus polymorphisms [42,55,56•,57]. NGS analysis of viruses obtained from the cattle transmission series described by Juleff and others [53] revealed that the dynamics of minority variants are consistent with genetic drift rather than strong selection and that viral population complexity is influenced by small intra-host bottlenecks and relatively large inter-host bottlenecks [56•].

## Conclusion

A spectrum of different mechanisms contributes to the transmission of FMDV at different scales. Uncertainty regarding the precise origins of FMD outbreaks and the specific connections between susceptible hosts, infected farms and different countries can be reduced by combining FMD virus sequence data with epidemiological data describing the spatial and temporal features of the outbreaks. Reconstructing patterns of FMD virus spread has the potential to help define and understand the risks for onward FMD transmission providing new focus for disease control initiatives such as targeted vaccination.

## Conflicts of interest

None.

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