**Supplementary Information**

**O1 FMDV-HS receptor complex**

An important factor which determines the infectivity of a number of animal viruses is the presence of suitable cellular surface receptors for attachment and internalization. The epithelial cell expressed heterodimer, integrin, has been shown to be the cellular receptor for FMDV *in vivo* (1-3). Although several integrins are known to bind to the conserved RGD amino acid motif found on the VP1 capsid protein of FMDV, including αvβ8 and αvβ3, the integrin αvβ6 is considered the main receptor of wild-type FMDV. However, (4) observed that the glycosaminoglycan, heparan sulphate (HS), could mediate the interaction of FMDV serotype O with cells in culture. Nine motifs, associated with the subtype O1 FMDV-HS receptor complex, namely residue 134, 135 and 138 of VP2, residues 56, 59, 60, 87 and 88 of VP3, and residue 195 of VP1 are discussed in (5). Amino acid residue 56 of VP3, an arginine in cell-culture-adapted viruses and commonly a histidine or cysteine in ‘field’ strains of FMDV, is critical to virus/receptor recognition (5, 6).

**Bottleneck Quantification Method**

We used the beta-binomial sampling method developed by (7), and applied in (8), to infer bottleneck sizes between parent and daughter samples; see (7) for full details. It is important to note that the method does not determine the number of viral genomes passing between parent and daughter, but the number of viral genomes that pass into the daughter and contribute genetically to the viral population that is being sequenced. The method is similar to a standard presence/absence model, but allows variant frequencies in the daughter host to change between the time of founding and the time of sampling (to account for the stochasticity of viral replication dynamics in the early stages of infection). The method is insensitive to the time interval between transmission and sampling, but determined by the size of the founding population (*Nb*) and the number of variant genomes *k* present in it. It is assumed that the variant frequency in the parent remains constant between sampling and transmission (8).

The likelihood of a transmission bottleneck size, *Nb*, given a variant frequency at genome position *i* is given by:

[Equation 1]

Where *VD,i* is the variant frequency at genome position *i* in the daughter and *p\_beta(VD,i | k, Nb - k)* is given by the beta probability function parameterized with the shape parameters *k* and *Nb-k,* and evaluated at *VD,i*. The term *p\_bin(k | Nb, VP,i)* denotes the binomial distribution evaluated at *k* and parameterized with *Nb* number of trials and a success probability of *VP,i*, where *VP,i* is the variant frequency at genome position *i* in the parent. In summary, this is the probability density that the transmitted variant is found in the daughter at a frequency *VD,i* given that the variant was observed in *k* genomes in a founding population of size *Nb* times the probability of drawing *k* variant genomes in a sample size of *Nb* and a variant frequency of *VP,i* in the parent, summed for all possible *k* (8).

If a parent variant at genome position *i* is not detected in the daughter, this could be because it is truly absent from the daughter or because it falls below the variant calling threshold, set to 0.5% for FMDV data based on our control studies (9). To allow for both these possibilities the likelihood that the transmission bottleneck size is *Nb*, given that the variant at genome position *i*was not detected is given by:

[Equation 2]

Where *T* is the variant calling threshold and *p\_beta\_cdf(VD,i < T|k, Nb – k)* is given by the beta cumulative distribution function at the variant calling threshold (7). We report the maximum likelihood estimates (MLE) of bottlenecks linking each pair of parent-daughter populations with associated 95% confidence intervals.

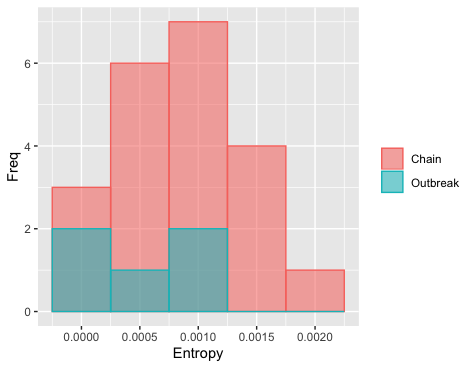
**In vitro bottleneck quantification results**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Bottleneck (viral genomes)** | |
| **Parent** | **Daughter** | **Population L** | **Population S** |
| Initial | P1 | 18846 [7178-39194] | 12652 [4828-26297] |
| P1 | P2 | 5221 [1871-11373] | 429 [178-883] |
| P2 | P3 | 140 [94-202] | 84 [49-139] |

**Table S1: Estimated bottleneck sizes for *in vitro* passage series.** Bottlenecks were determined using the methodology of (7), with the MLE of bottleneck size shown, with 95% confidence intervals shown in square brackets.

**Entropy**

We compared the Shannon entropy present of samples from the *in vitro* transmission chain (21 samples), to those from the 2001 outbreak of FMDV (5 samples). We used Shannon entropy formula from (10) (Fig. 7), which only considers mutations above the 0.5% cutoff. From the figure it is clear that the entropies from the samples from the chain infected with a recently passaged virus are no lower than those natural populations circulating in the UK in 2001.



**References**

1. Jackson T, Clark S, Berryman S, Burman A, Cambier S, Mu D, et al. Integrin alphavbeta8 functions as a receptor for foot-and-mouth disease virus: role of the beta-chain cytodomain in integrin-mediated infection. J Virol. 2004;78(9):4533-40.

2. Monaghan P, Gold S, Simpson J, Zhang Z, Weinreb PH, Violette SM, et al. The alpha(v)beta6 integrin receptor for Foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. J Gen Virol. 2005;86(Pt 10):2769-80.

3. O'Donnell V, Pacheco JM, Gregg D, Baxt B. Analysis of foot-and-mouth disease virus integrin receptor expression in tissues from naive and infected cattle. J Comp Pathol. 2009;141(2-3):98-112.

4. Jackson T, Ellard FM, Ghazaleh RA, Brookes SM, Blakemore WE, Corteyn AH, et al. Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. J Virol. 1996;70(8):5282-7.

5. Fry EE, Lea SM, Jackson T, Newman JW, Ellard FM, Blakemore WE, et al. The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. EMBO J. 1999;18(3):543-54.

6. Borca MV, Pacheco JM, Holinka LG, Carrillo C, Hartwig E, Garriga D, et al. Role of arginine-56 within the structural protein VP3 of foot-and-mouth disease virus (FMDV) O1 Campos in virus virulence. Virology. 2012;422(1):37-45.

7. Sobel Leonard A, Weissman DB, Greenbaum B, Ghedin E, Koelle K. Transmission Bottleneck Size Estimation from Pathogen Deep-Sequencing Data, with an Application to Human Influenza A Virus. J Virol. 2017;91(14).

8. McCrone JT, Woods RJ, Martin ET, Malosh RE, Monto AS, Lauring AS. Stochastic processes constrain the within and between host evolution of influenza virus. Elife. 2018;7.

9. Orton RJ, Wright CF, Morelli MJ, King DJ, Paton DJ, King DP, et al. Distinguishing low frequency mutations from RT-PCR and sequence errors in viral deep sequencing data. BMC Genomics. 2015;16:229.

10. Morelli MJ, Wright CF, Knowles NJ, Juleff N, Paton DJ, King DP, et al. Evolution of foot-and-mouth disease virus intra-sample sequence diversity during serial transmission in bovine hosts. Vet Res. 2013;44:12.