Supplementary methods

Generation of recombinant FMD viruses

For the generation of a recombinant FMD virus containing the capsid-coding P1 region of O/BUL/HS018-1/2011 in an O/FRA/1/2001 backbone, a restriction-free cloning approach [1] was used. The P1 region of the O/BUL/HS018-1/2011 virus isolate was amplified by RT-PCR after RNA extraction from cell supernatant using the QIAamp Viral RNA Kit (QIAGEN). A megaprimer of the P1 region with overhangs matching O/FRA/1/2001 was generated using AMV Reverse Transcriptase (NEB) and Phusion Hot Start Flex DNA Polymerase with the following primers: OBUL-P1-Fwd 5' AAGCA AAGGT TCAGA AACGA CTCAG AGGTG CTGGG CAATC CAGC 3' and OBUL-P1-Rev 5' GACCT GACGT CAGAG AAGAA GAAAG GCCCA GGGTT GGACT CAAC 3'. In a second PCR, again using Phusion polymerase, the P1-megaprimer was combined with the pT7S3-O FRA plasmid in a 1:20 molar ratio. The PCR product was incubated with DpnI for 2 h at 37 °C to digest any leftover template DNA and then transformed into competent bacteria (NEB 10-beta Escherichia coli). BSR-T7 cells [2] in a 24-well plate were transfected with purified plasmid DNA. Each well was transfected with 500 ng of DNA using 1.5 µl of Lipofectamine 3000. For this purpose, 30 plasmids with the correct restriction fragment pattern were constructed, and after multiple rounds of transfections, three infectious clones of recombinant O/FRA/2001-P1(O/BUL/2011) were obtained. The rescued virus was subsequently passaged twice on BHK-21 cells. For each passage, 250 µl of clarified cell lysate obtained by freezing and thawing was used to infect fresh cells. CPE was observed from the second passage onwards. All virus stocks were sequenced using Sanger sequencing [3] and were stored at -80 °C. Virus titers were determined by titration on BHK-21 cells and read after 48 and 72 hours. Tissue culture infectious doses ($TCID_{50}$) were calculated using the Spearman-Kaerber formula.

References

1. Bond SR, Naus CC: **RF-Cloning.org: an online tool for the design of restriction-free cloning projects.** *Nucleic acids research* 2012, **40**(Web Server issue):W209-13.

2. Buchholz UJ, Finke S, Conzelmann KK: **Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter.** *Journal of virology* 1999, **73**(1):251–259.

3. Dill V, Beer M, Hoffmann B: **Simple, quick and cost-efficient: A universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses.** *Journal of virological methods* 2017, **246**:58–64.