FMD OUTBREAKS IN EU IN 2025:

THE USE OF SEQUENCING (SANGER AND FULL GENOME)

**STANDING COMMITTEE ON PLANTS, ANIMALS,
FOOD AND FEED (PAFF)**
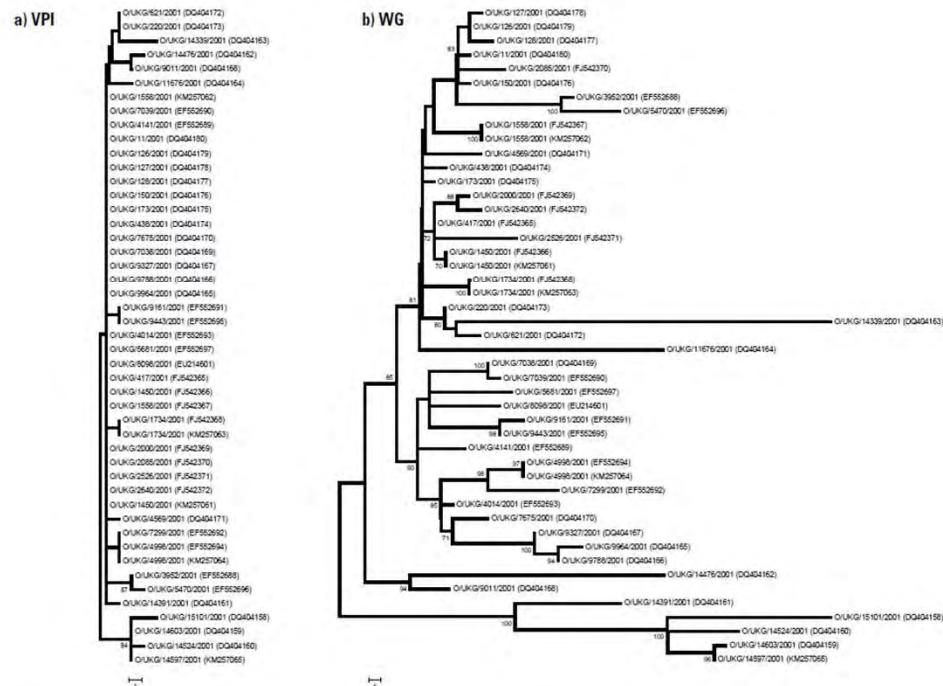21 May 2025

FGS vs VP1 sequencing : advantages

Genomics and outbreaks: foot and mouth disease

G.L. Freimanis, A. Di Nardo, K. Bankowska, D.J. King, J. Wadsworth, N.J. Knowles & D.P. King*

Fig. 4
A comparison between phylogenetic analysis of data generated from the same foot and mouth disease virus isolates collected during the 2001 foot and mouth disease epidemic in the United Kingdom using VP1 only or WG (whole genome)
Maximum likelihood trees were produced using MEGAv.6.06 (70)

With access to the full genome, you increase the resolution and you can find more differences between isolates that can be identical based on VP1 analysis

Previous usage of FGS for FMDV (UK 2001)

Molecular Epidemiology of the Foot-and-Mouth Disease Virus Outbreak in the United Kingdom in 2001[▽]

Eleanor M. Cottam,[1,2]* Daniel T. Haydon,[2] David J. Paton,[1] John Gloster,[4]† John W. Wilesmith,[3] Nigel P. Ferris,[1] Geoff H. Hutchings,[1] and Donald P. King[1]

TCS statistical parsimony analysis clearly showed the genetic evolutionary history of the virus.

Transmission routes can be determined by traditional contact tracing (black arrows) and by genetic data (gray arrows) (slight differences can be observed)

During a 7-month period (2001 FMDV outbreak in UK), 197 nt substitutions at 191 different sites

Nucleotide change corresponding to 0.00825 substitutions/site/year = 0.9% of the genome is predicted to change per annum
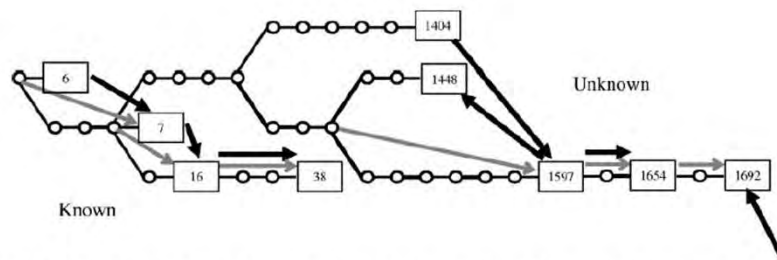
FIG. 5. TCS analysis of two clusters of infected premises. Statistical parsimony analysis of known and unknown virus transmission events. Each connecting branch line represents a nucleotide substitution, with each dot representing a putative ancestor virus. Black arrows indicate the transmission route determined by traditional contact tracing, gray arrows indicate the route supported by the genetic data. The data show the close genetic relationship of IP1692 to IP1597 and IP1654, although epidemiological tracing suggested IP1692 was infected from a more distantly related source.

Previous usage of FGS for FMDV (Bulgaria 2011)

Reconstruction of the Transmission History of RNA Virus Outbreaks Using Full Genome Sequences: Foot-and-Mouth Disease Virus in Bulgaria in 2011

Begoña Valdazo-González[1]*, Lilyana Polihronova[2], Tsviatko Alexandrov[3], Preben Normann[4], Nick J. Knowles[1], Jef M. Hammond[1], Georgi K. Georgiev[3], Fuat Özyörük[5], Keith J. Sumption[6], Graham J. Belsham[4], Donald P. King[1]

Figure 3. Statistical parsimony trees as implemented by TCS using the full genomes of 19 sequenced FMDVs. A. Edited TCS tree in which putative virus ancestors (○), except those corresponding to nodes, were removed. The length of the branches is directly proportional to the number of nucleotide (nt) changes. The vertical axis represents a time scale which denotes the date when the viruses were collected. **B.** Detailed TCS tree showing the viruses corresponding to the Bulgarian outbreaks and their closest ancestor within the Middle East. Open circles and lines correspond to putative genetic intermediates separated by single nt changes. Putative common (red circle) and secondary ancestors for each wave are shaded (blue circle, first; green circle, second). Lines in bold correspond to non-synonymous changes. The square shows the number of nt versus non-synonymous changes. The specific amino-acid changes are indicated, as well as the viral proteins involved. Non-conservative amino-acid substitutions (according to GONNET matrix, as implemented in BioEdit software) are highlighted in bold.
doi:10.1371/journal.pone.0049650.g003

Conclusions of the paper FMDV in Bulgaria 2011:

The number of nucleotide substitutions that were present between, and within, these separate clusters provided evidence that **undetected FMDV infection had occurred** ➔ Long branches (more than 10 nt) may indicate the presence of unsampled cases.

Presence of a single putative common ancestor for all the sequences recovered from infected animals provides clear evidence for a single introduction of the virus.

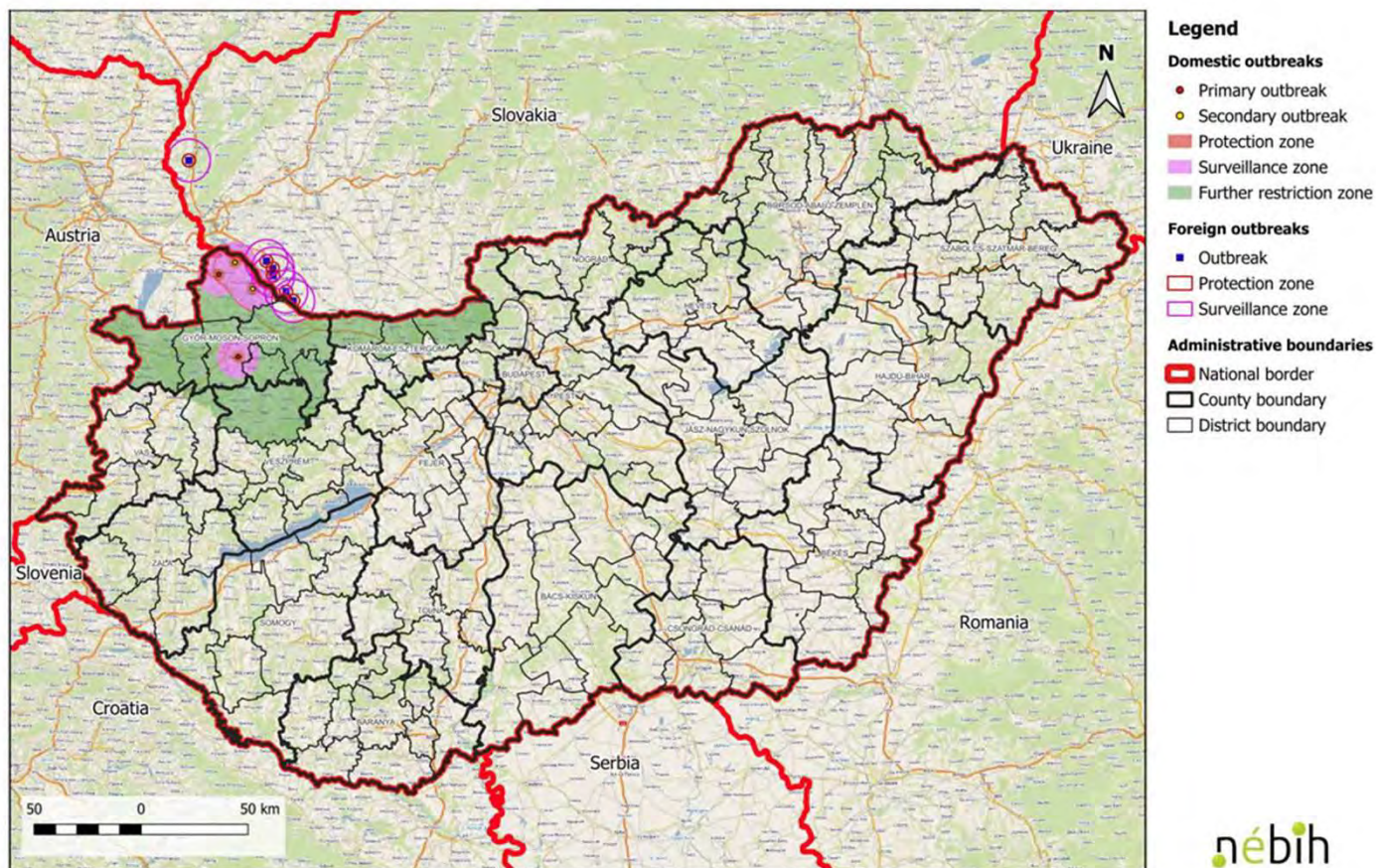The closest relative from outside of Bulgaria was a FMDV collected during 2010 in Bursa (Anatolia, Turkey) ➔ around 40 nt of difference

Full Genome Sequencing of EU cases in Hungary and Slovakia

FMDV situation in EU (April 2025)

- 5 outbreaks in HUN
- 6 outbreaks in SK

FGS for EU cases in Hungary and Slovakia (performed at EURL)

Country	Outbreak number	Date of confirmation of the outbreak	Number of animals in the farm	Nb of samples received	FGS performed
Hungary	H1 (Kisbajcs)	06/03/2025	1370	4	Yes (1/4)
Slovakia	S1 (Medvedov)	21/03/2025	706	3	Yes (2/3)
Slovakia	S2 (Narad)	21/03/2025	806	3	Yes (3/3)
Slovakia	S3 (Baka)	21/03/2025	1313	2	Yes (2/2)
Slovakia	S4 (Mala Luc)	25/03/2025	268	5	Yes (5/5)
Slovakia	S5 (Plavecky Stvrtok)	30/03/2025	3521	4	Yes (4/4)
Hungary	H2 (Levél)	26/03/2025	2573	3	Yes (3/3)
Hungary	H3 (Darnózseli)	02/04/2025	1051	2	Yes (2/2)
Hungary	H4 (Dunakiliti)	02/04/2025	2597	1	Yes (1/1)
Hungary	H5 (Rábapordány)	17/04/2025	875	2	Yes (2/2)
Slovakia	S6 (Jurova)	04/04/2025	876	8	Yes (8/8)

TCS tree (statistical parsimony analysis)

Presence of a single putative common ancestor for all the sequences recovered from infected animals provides evidence for a **single introduction of the virus**.

Putative secondary ancestors for both « sub-branches »

Each branch = 1 nt of difference

S5 3521 ans
SLO2803_Plavecky_Stvrok_30/03/25
SLO2805_Plavecky_Stvrok_31/03/25
SLO2806_Plavecky_Stvrok_30/03/25

S5
SLO2804_Plavecky_Stvrok_31/03/25

S1
SLO2795_Narad_20/03/25

S1 806 ans
SLO2793_Narad_20/03/25
SLO2794_Narad_20/03/25

H3 1051 ans
HUN3065_Darnozseli_01/04/25
HUN3066_Darnozseli_01/04/25

2597 ans H4
HUN3067_Dunakiliti_01/04/25

HUN3266_Rabapordany_17/04/25
HUN3267_Rabapordany_17/04/25

H5 875 ans
SLO2801_Mala_Luc_26/03/25
SLO2802_Mala_Luc_26/03/25

SLO2798_Mala_Luc_24/03/25
SLO2799_Mala_Luc_26/03/25
SLO2800_Mala_Luc_26/03/25

S4 268 ans

S1 706 ans
SLO2791_Medvedov_20/03/25
SLO2792_Medvedov_20/03/25

PAK_C8_2018

87 nt

H2
HUN3064_Level_25/03/25

H2 2573 ans
HUN3062_Level_25/03/25

H2
HUN3063_Level_25/03/25

H1 1370 ans
HUN2060_Kisbajcs_06/03/25

S6
SLO3319_Jurova_09/04/25

S6 876 ans
SLO3321_Jurova_09/04/25
SLO3322_Jurova_09/04/25

SLO3320_Jurova_09/04/25 **S6**

SLO3318_Jurova_09/04/25 **S6**

S1 1313 ans
SLO2796_Baka_20/03/25
SLO2797_Baka_20/03/25

SLO3316_Jurova_09/04/25 **S6**

SLO3315_Jurova_09/04/25 **S6**

SLO3317_Jurova_09/04/25 **S6**

▲ Samples from same animals

Comparison based on 7739 nt due to « near complete » FGS of PAK C8 2018 (EU FGS are around 8180 nt)

Conclusions

Take-home messages

- **FGS give a picture of the outbreaks related to available data** (Not all samples have been retrieved from all animals, and differences between apparition of clinical signs on animals and sampling lead to virus replication, and to mutations : **Full genome analyses of 33 samples** (9 from Hungary and 24 from Slovakia) **was performed at EURL**

➜ Single introduction of the virus

➜ "Real" ancestor of the outbreak not sampled (probably originated from Hungary few days before the sampling of the first animal; date of first infection was estimated 27-28 February 2025 (EUVET report))

➜ No evidence of unsampled farm

- To have the "perfect" picture of the outbreaks, sequencing of all animals (or a large majority) could have help (but very difficult in time of outbreaks, as culling of animals and containing dissemination of the virus is the priority)

- More investigation is required, especially with the Turkish strain identified in the VP1 phylogenetic tree (TUR/Gaziantep/2024) ➜ isolates will be sent to EURL soon

Thank you for your attention