

Genome Sequencing of Foot-and-Mouth Disease Virus Type O Isolate GRE/23/94

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The complete genome of a foot-and-mouth disease type O virus originating from an epidemic in Greece in 1994 is reported. This virus belongs to the Middle East-South Asia topotype.

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Foot-and-mouth disease (FMD) virus belongs to the genus *Aphthovirus* within the family *Picornaviridae* and causes FMD in cloven-hooved animals. FMD virus has a positive-sense single-stranded RNA genome of approximately 8.3 kb, which contains a single open reading frame (ORF) encoding 4 structural and 10 nonstructural proteins (1).

The 1994 FMD virus (FMDV) epidemic in Greece began with high morbidity in sheep. After approximately 1 month there was a sharp decline of clinical cases within sheep flocks, while morbidity within cattle and pig populations remained stable. There is evidence to suggest that FMDV cannot maintain itself within sheep populations for long periods of time (2), and therefore the full genome of a representative field isolate (GRE/23/94) was determined to promote understanding of whether there were any genomic determinates of host specificity for these outbreaks.

O/GRE/23/94 was obtained from the World Reference Laboratory for FMD (WRLFMD) collection (The Pirbright Institute, United Kingdom) and was isolated, using primary calf thyroid cells, from ovine epithelium collected on 4 August 1994 in Xanthi during the peak of the Greek FMD epidemic (2). RNA was extracted using an RNeasy minikit (Qiagen) and Illumina sequencing was performed as previously described (3). Raw FASTQ files were filtered using Sickle (4), with reads below a quality score of Q30 and a length of 25 nucleotides removed. Consensus sequences were generated using the *de novo* assembler program IDBA UD (version 1.1.1) (5), with an optimum k-mer length determined within the program. FMDV sequences were identified from the contigs produced by a BLAST search and assembled using BioEdit (version 7.2.5). Using the generated consensus sequence as a reference, BWA-MEM (version 0.7.11) (6) was used for alignment while SAMtools (version 1.3) (7) was used to process SAM/BAM files. Alignments were visually checked using Tablet (8), coverage plots were generated using BEDTools (version 2.25) (9), and a final consensus sequence was generated by use of DiversiTools (<https://github.com/josephhughes/btctools>). The total length of the genome recovered was 8,161 nucleotides with a mean coverage depth of 1,634 across the genome; we were not able to determine the sequence or length of the poly(C) tract or 7 adjacent downstream nucleotides. Comparison of O/GRE/23/94

with nine sequences belonging to the Middle East-South Asia topotype (10) (AJ539136, AJ539138, AJ539141, AY593823, AY593828, DQ404171, HQ268524, JX040493, and KT003716) showed no indels within the polyprotein ORF; however, a deletion of 43 nucleotides within the 5' untranslated region (located at position 410) was shared with AY593828 and KT003716, relative to the other seven sequences. We hypothesize that this represents the deletion of one of four pseudoknots of unknown function commonly present in other FMDV genomes. Additionally, a cluster of seven amino acid substitutions, different from the nine other sequences, was found in the carboxy-terminal half of the 3A polypeptide, which has been implicated in host specificity (11–13). The VP1 sequence of O/GRE/23/94 was identical to that previously reported (14).

Nucleotide sequence accession number. The nucleotide sequence for FMDV O/GRE/23/94 has been deposited in GenBank under accession number [KU726614](https://www.ncbi.nlm.nih.gov/nuccore/KU726614).

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