

# Phylogenetic Comparison of the Capsid-coding Region of All Seven Foot-and-Mouth Disease Virus Serotypes



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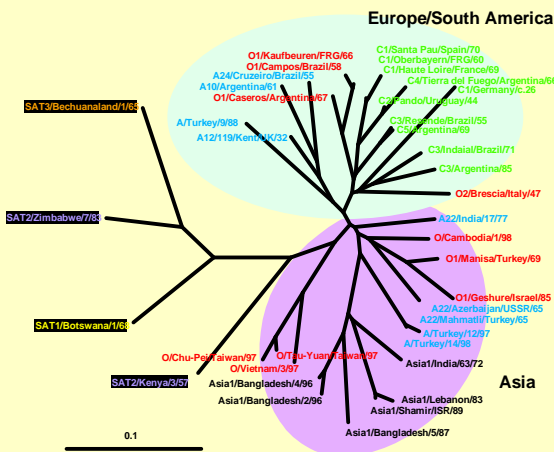
## ABSTRACT

We have determined the capsid-coding sequences of 12 foot-and-mouth disease viruses: three serotype O, five serotype A, one serotype Asia 1, one serotype SAT1 and two serotype SAT2. Nucleotide sequences were aligned with 33 previously published capsid sequences and phylogenetic analyses were performed. Neighbor-joining trees were constructed for the P1, 1A (VP4), 1B (VP2), 1C (VP3) and 1D (VP1) regions. Additionally trees were determined for parts of VP1 (G-H loop region and 3' end) to assess their use in phylogenetic analyses. A limited set of previously published sequence data for regions outside the capsid was also analysed (only serotypes O, A, C, SAT2). Phylogenetic trees for the outer capsid polypeptide-coding genes grouped the viruses according to their serotype. However, VP4 showed little correlation with serotype and tended to group viruses by their geographic origin. Similarly, the L, P2, 2C, 3C, 3D coding regions and the 5' and 3' untranslated regions showed no correlation with serotype, however, the data-sets were too limited to determine if there was any correlation with geographic origin. There are two theories which could explain the lack of serotype-specificity outside the capsid protein-coding region and also allow some degree of correlation with geographic origin; i) intertypic recombination; or ii) a low limit of allowed sequence variation, presumably controlled at the protein level. It is very likely that recombination takes place in the field. However, it would either have to be very common or the recombinant strains would have to become geographically widespread (within a particular region) to produce the patterns we observe.

## INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most economically important diseases of domestic livestock principally due to its highly contagious nature. The species *Foot-and-mouth disease virus* (FMDV) is a member of the genus *Aphthovirus* in the family *Picornaviridae* and consists of seven immunologically distinct serotypes: O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2 and SAT 3. We have determined the sequences of 12 capsid-coding regions and compared them to previously published sequence data and constructed phylogenetic trees containing representatives of all seven serotypes.

Fig. 2. Neighbor-joining tree showing the relationships between the VP4-coding regions of 39 foot-and-mouth disease viruses.



## REFERENCES

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- Page, R.D.M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357-358.
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- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Fig. 4. Neighbor-joining tree showing the relationships between the 3C protease coding regions of 11 foot-and-mouth disease viruses.

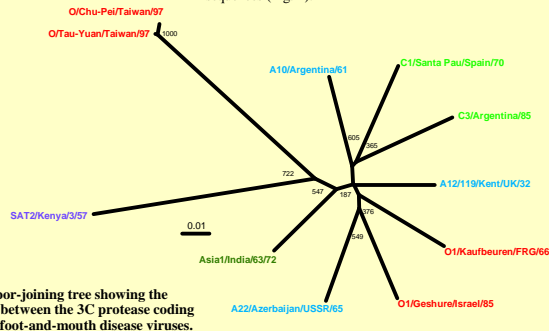
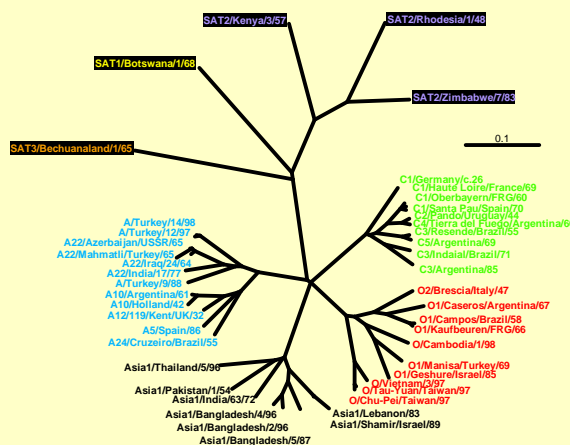


Fig. 1. Neighbor-joining tree showing the relationships between the capsid-coding regions (P1) of 45 foot-and-mouth disease viruses.



## MATERIALS AND METHODS

The FMD viruses examined are listed in Table 1 along with their database accession numbers (where available) and references.

The sequences were determined using one of two methods; either molecular cloning followed by conventional dideoxy-sequencing using M13 primers (A24/Cruzeiro/Brazil/55, SAT2/Kenya/3/57, SAT2/Rhodesia/1/48) or by RT-PCR followed by cycle sequencing using a variety of primers (O1/Manisa/Turkey/69, O/Vietnam/3/97, O/Cambodia/1/98, A22/Mahmatli/Turkey/65, A/Turkey/9/88, A/Turkey/12/97, A/Turkey/14/98, Asia1/Pakistan/1/54, SAT1/Botswana/1/68). These primer sequences are available on request.

Sequence alignments were performed using BioEdit v.4.8.6 (Hall, 1999) and Clustal W v.1.8 (Thompson et al., 1994) and phylogenetic analyses carried out using Clustal X v.1.8 (Thompson et al., 1997). The trees were plotted using TreeView 1.6 (Page, 1996).

## RESULTS AND DISCUSSION

Fig. 1 shows a Neighbor-joining tree comparing the capsid-coding regions of the 45 FMD viruses studied. The virus strains clustered according to serotype. Similar analyses of the VP1, VP2 and VP3 coding genes gave identical trees (data not shown). However, no serotype-specific clustering was seen in the tree for the VP4 (in inner capsid polypeptide) gene (Fig. 2). Instead, for serotypes O and A, viruses cluster into two major groups; those coming from Europe or South America and those from Asia. It is known that South American viruses originated from Europe (the first introduction was in 1870). Viruses belonging to the Asia 1 serotype formed a single lineage. This may be because they are have a recent common ancestor. A more diverse group of these viruses requires investigation. No Asian type C viruses have yet been studied.

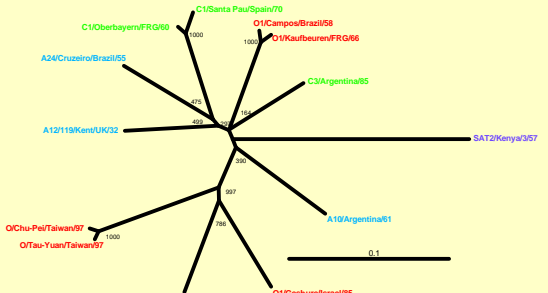
Only a limited number of SAT viruses were studied, therefore it is not clear if the grouping of the southern African viruses away from the single East African SAT 2 virus is a pattern which will be confirmed on examination of further virus sequences (Fig. 2).

Table 1. The origin of foot-and-mouth disease virus sequences compared in this study.

Virus designation	Database accession no.	Reference
O1/Campos/Brazil/58	M95781	Jensen and Moore, 1993
O1/Kaufbeuren/FRG/66	X00871	Fors et al., 1984
O1/Caseros/Argentina/67	U82271	Tani et al., 1997
O2/Brescia/Italy/47	M55287	Krebs et al., 1991
O1/Manisa/Turkey/69	AJ251477	This work
O1/Geshure/Israel/85	AF189157	Benvenisti et al., unpub.
O/Vietnam/3/97	na	This work
O/Tau-Yuan/Taiwan/97	AF154271	Kuo et al., unpublished
O/Chu-Pei/Taiwan/97	AF026168	Liu and Wu, unpublished
O/Cambodia/1/98	na	This work
A5/Spain/86	M72587	Saiz et al., 1991
A10/Holland/42*	M20715-17	Thomas et al., 1988
A10/Argentina/61 (A61)	X00429	Carroll et al., 1984
A12/119/Kent/UK/32	M10975	Robertson et al., 1985
A22/Iraq/24/64	AJ251474	Bolwell et al., 1989
A22/Azerbaijan/USSR/65	X74812	Sosnovtsev et al., unpub.
A22/Mahmatli/Turkey/65	na	This work
O2/Brescia/Italy/47	na	This work
O1/Caseros/Argentina/67	na	Tosh et al., 2000
A24/Cruzeiro/Brazil/55	AJ251476	This work
A/Turkey/9/88	na	This work
A/Turkey/12/97	na	This work
A/Turkey/14/98	na	This work
C1/Germany/c.26	M90368	Martinez et al., 1992
C1/Oberbayern/FRG/60	X00130	Beck et al., 1983
C1/Santa Pau/Spain/70 (C-S8)	A1133557	Toja et al., 1999
C1/Haute Loire/France/69	L29061	Mateu et al., 1994
C2/Pando/Uruguay/44	M90367	Martinez et al., 1992
C3/Ressende/Brazil/55	M90381	Martinez et al., 1992
C3/Indiaia/Brazil/71	M90376	Martinez et al., 1992
C3/Argentina/85	AJ007347	Escarmis et al., 1998
C4/Tierra del Fuego/Argentina/66	M90372	Martinez et al., 1992
C5/Argentina/69	L29078	Mateu et al., 1994
Asia1/Lebanon/83	U01207	Stram et al., 1994
Asia1/India/63/72	Y09949	Reddy et al., 1999
Asia1/Pakistan/1/54*	AJ251478	This work
Asia1/Bangladesh/5/87	na	Marquardt et al., 2000
Asia1/Shamir/Israel/89	na	Marquardt et al., 2000
Asia1/Bangladesh/2/96	na	Marquardt et al., 2000
Asia1/Bangladesh/4/96	na	Marquardt et al., 2000
Asia1/Thailand/5/96*	na	Marquardt et al., 2000
SAT1/Botswana/1/68	Z98203	This work
SAT2/Kenya/3/57	AJ251473	This work
SAT2/Rhodesia/1/48	AJ251475	This work
SAT2/Zimbabwe/7/83	AF136607	Van Rensburg and Nel, 1999
SAT3/Bechuana/land/1/65	M28719	Brown et al., 1989

\* the sequence of VP4 is only partial or not available  
na, not available

Fig. 3. Neighbor-joining tree showing the relationships between the leader (L) coding regions of 13 foot-and-mouth disease viruses.



## EXAMINATION OF PARTIAL VP1 SEQUENCES

Neighbor-joining trees were constructed using the genome regions coding for i) the VP1 GH loop and ii) the VP1 3' 170 nucleotides (data not shown). In both cases, as with the complete VP1 tree, sequence clustering correlated with serotype. However, in the GH loop-coding tree the FMDV C3-related viruses clustered together in one group, whereas in the P1/VP1/VP1 3' end trees they formed two distinct lineages, C3/Ressende-like and C3/Indiaia-like. The relationship evident in the GH loop tree may reflect antigenic similarities which do not have a phylogenetic basis. Therefore the use of sequences in which the GH loop-coding region is a significant part may give misleading results.

## EXAMINATION OF THE NON-STRUCTURAL-CODING AND UNTRANSLATED REGIONS

Examination of a small number of sequences of the FMDV leader (Fig. 3), 2C (data not shown), 3C (Fig. 4) and 3D (data not shown) genes gave essentially identical trees and showed no correlation with serotype. Similarly, analysis of partial 5' untranslated region (UTR) and complete 3' UTR sequences gave the same result.