Genetic Relationships Between Recent and Historical Isolates of Foot-and-Mouth Disease Viruses From South America

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INTRODUCTION

Although a number of South American foot-and-mouth disease viruses (FMDV) have been examined by nucleotide sequencing of their VP1 genes (e.g. Piccone et al., 1988; Martínez et al., 1992; Maliirat et al., 1994; Mateu et al., 1996), there have been few attempts to study the moleculer epidemiology of FMD in this region. The ability to trace the origin of outbreaks of disease is important to the application of control measures in the field. The accurate prediction of virus origin is often dependant on having a large database of sequences for the geographic region under study as well as for other more distant geographic regions. The World Reference Laboratory for FMD (WRLFMD), based at the IAH-Pirbright, has already built a large nucleotide sequence database for many regions around the world. We report here, a collaborative study in which we have begun to build such a database for FMD viruses from South America.

MATERIALS AND METHODS

Viral RNA was extracted either with TRIzol reagent (Gibco-Life Technologies) or using RNeasy spin columns (Qiagen) according to the manufacturers' protocols. RNA was reverse transcribed into cDNA using either random primers (Promega) or a specific FMDV primer (NK61) and 200 U of MMLV-RT (Promega). The PCR was performed using different primers for each serotype of FMDV, traditionally used by the WRLFMD, in combination with an "antisense" universal primer located in the 2B gene (NK61; Knowles and Samuel, 1994, 1998). The purified PCR products were sequenced using the fmol sequencing system (Promega) and the sequences were aligned manually. Phylogenetic analysis was performed using programs from the PHYLIP package (Felsenstein, 1993) and EpiSeq programs (N.J. Knowles, unpub.). The trees were drawn using TreeView (Page, 1996). For comparative purposes, we included previously reported VP1 sequences of South American isolates and some European strains.

Fig. 1. Neighbor-joining tree showing the relationships between the VP1 genes of the FMD type O viruses studied. The sequences of viruses labelled in red were determined in this study. Those in purple were kindly provided by Dr. Ingrid Bergmann. Others were obtained from the EMBL database.

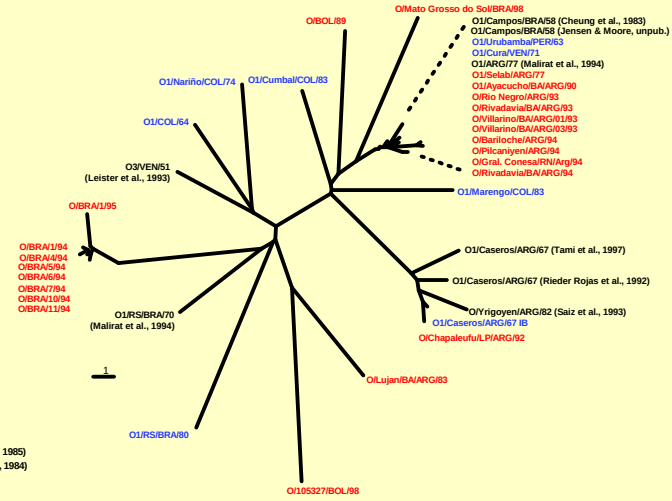
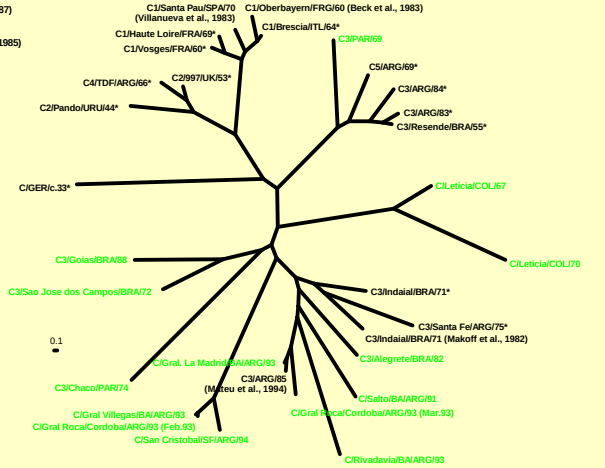


Fig. 2. Neighbor-joining tree showing the relationships between the VP1 genes of the FMD type A viruses studied. The sequences of viruses labelled in blue were determined in this study. Others were obtained from the EMBL database.

Fig. 3. Neighbor-joining tree showing the relationships between the VP1 genes of the FMD type C viruses studied. The sequences of viruses labelled in green were determined in this study. Others were obtained from the EMBL database. * Sequences from Martínez et al., 1992



RESULTS AND DISCUSSION

Complete or partial VP1 sequences were determined for 23 type O, 40 type A and 14 type C foot-and-mouth disease (FMD) viruses isolated between 1959 and 1998 from outbreaks in various countries in South America.

FMD TYPE O

Eight type O viruses isolated in 1994 and 1995 from the State of Minas Gerias, Brazil formed a lineage distinct from all other type O viruses, but was most closely related to O1/RS/Bra/70. Another Brazilian type O virus isolated in Mato Grosso do Sul in 1998 was distantly related to O1/Campos/Brazil/58 (~10% nucleotide difference). A Bolivian type O virus from 1998 also formed a distinct lineage (Fig. 1).

The last outbreaks of FMDV type O in Argentina occurred in 1993-94 in the Rio Negro, La Pampa and Buenos Aires provinces. Phylogenetic analysis showed that most of the viruses isolated between 1990 and 1993 were closely related to O1/Campos/Bra/58, a vaccine virus strain commonly used in many South American countries. Another virus isolated in 1992 was closely related to O1/Caseros/Arg/67, the main vaccine strain used in Argentina. One virus isolated in 1983 formed a distinct lineage (Fig. 1).

FMD TYPE A

Phylogenetic analysis of the type A FMD viruses revealed more complex relationships. Two viruses isolated from Minas Gerias, Brazil in 1994 (A/BRA/12/94) and 1995 (A/BRA/3/95) along with viruses from Rio Grande do Sul, Brazil in 1997 and Bolivia in 1998 formed a distinct lineage, although considerable variation was observed between most of the individual virus isolates. Two other viruses from Minas Gerias, Brazil in 1993 (A/BRA/10/93 and A/BRA/11/93) were closely related to each other, but formed a lineage distinct from all the other type A viruses. Three viruses isolated in Colombia in 1997 belonged to a lineage which included A24/Cruzeiro/Brazil/55 and A32/Venezuela/70, however, they formed their own clade and showed some variation to each other. A single type A isolate from Colombia in 1998 formed a lineage distinct from all the others studied (Fig. 2).

Virus isolates causing outbreaks in Argentina in 1990-92 were most closely related to Argentinean and Uruguayan field strains from 1979 to 1987; however, considerable variation was observed between most of the isolates. Brazilian type A viruses from 1980s were generally distinct from those from Argentina and fell into a number of different lineages. The Argentinean vaccine production strain A/Arg/79 was most closely related to A/Venceslau/Brazil/76 (Fig. 2).

In the case of the type A viruses there were no isolates which were closely related to any vaccine virus strains.

FMD TYPE C

Six type C viruses isolated from Argentina between 1991 and 1994 were compared with seven older viruses from Brazil, Colombia and Paraguay and well as a number of previously published sequences (Fig. 3).

Outbreaks of FMDV type C occurred in Buenos Aires and Santa Fe provinces in 1993-94 (Feigelstock et al., 1996). Some of these viruses were closely related to the vaccine strain C3/Arg/85 (e.g. C/General La Madrid/BA/Arg/93), however, others were quite distinct (e.g. C/General Villegas/BA/Arg/93) suggesting the possibility of muliplte origins for these outbreaks. C3/Resende/Bra/55, C5/Arg/69, C3/Paraguay/69 and viruses isolated from an epizootic outbreak in Argentina in 1983-84, formed another distinct lineage. C4/Tierra del Fuego/Arg/66 differed considerably from any other Argentinian type C examined, and it defines a separate lineage that also contains isolates from Uruguay and the United Kingdom, C2/Pando/Uruguay/44 and C2/997/UK/53. Two viruses from Colombia (1967 and 1970) formed another disinct lineage (Fig. 3).

CONCLUSIONS

A better understanding of the precise origins/relationships between FMD viruses isolated from outbreaks in South America will require the study, by nucleotide sequencing, of many more recent and historical field isolates. The laboratories involved in this study are now collaborating to build such a database.

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