Global Molecular Epidemiology of Foot-and-Mouth Disease Virus Type C

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ABSTRACT

The epidemiology of foot-and-mouth disease (FMD) serotype C is not well understood. In the past outbreaks due to this serotype in South America, Africa and Asia were far less prevalent than the other serotypes which occur in these regions. Since 1995, less than ten suspected outbreaks of FMDV C have been reported throughout the world. Laboratory confirmation has been obtained for cases in Nepal in 1996, Kenya in 1996, 1998, 2000 and 2004, and Brazil in 2004. A suspected outbreak in Pakistan in 2004 has not been confirmed. To extend our knowledge of the epidemiology of FMDV C the complete VP1 sequences of 101 isolates from Europe, South America, Asia and Africa were determined and compared with those already published. Phylogenetic analyses revealed four major lineages. The first contained the classical C_1 and C_2 subtypes which were found in mainly in Europe. The second was one of two C_3 lineages which occurred almost exclusively in South America, apart from one isolate from Angola in Africa. The third lineage contained the other South American C_3 lineage (as represented by C_3/Resende/Brazil/55) with additional isolates from Europe and the Middle East from the period 1969-70. This lineage also contained isolates from the Philippines (1976 to 2004). The fourth major lineage contained viruses from Asia (Bangladesh, Bhutan, India, Kuwait, Nepal, Saudi Arabia, Sri Lanka and Tajikistan) and Africa (Ethiopia, Kenya and Uganda). The probable re-introduction of laboratory or vaccine virus strains into the field was evident in many cases, particularly in Europe.

INTRODUCTION

Historically foot-and-mouth disease virus (FMDV) type C has had a narrow geographic distribution compared to either type O or type A. It has principally been found in Europe and South America. FMD was first introduced into South America from Europe in 1870, but it is not known when type C first reached that continent. FMDV C has only been recorded in sub-Saharan Africa in Kenya (from 1957), Ethiopia (from 1957) and Uganda (from 1970). In North Africa and the Middle East only sporadic outbreaks have been reported: many of which remain unconfirmed. In the Indian sub-continent, FMDV C has been recorded since 1954. FMDV C had not been recorded in Southeast Asia until it was introduced into the Philippines in 1976 where it persisted until at least 2004.

Few studies have targeted FMDV C for molecular epidemiological studies (Piccone *et al.*, 1988; Knowles & Samuel, 1988, 1990; Knowles, 1990; Martínez *et al.*, 1992; Konig *et al.*, 2001; Hemadri *et al.*, 2003; Nagendrakumar *et al.*, 2005). Therefore, in this study we have sequenced the complete VP1-coding region of 101 FMDV C isolates in an attempt to better understand the epidemiology of this serotype.

MATERIALS & METHODS

Primers

Four alternative primer combinations were used for the RT-PCR: C-536F/NK61, C-536F/EUR-2B52R, C-1C616F/NK61 and C-1C616F/EUR-2B52R (Table 1). Amplicon sizes for the first two sets were 877 to 883 bp (depending on the virus isolate) and 796 to 802 bp for the second two sets. Forward and reverse primer amounts were 20 and 40 pmoles, respectively. Two to three sequencing primers were used to ensure coverage of the VP1 region on both DNA strands (Table 1).

RT-PCR or vRNA

Total RNA was extracted from 460 µl of a 10% epithelial suspension or cell culture supernatant using RNeasy™ kits (Qiagen) according to the manufacturer's instructions and resuspended in 50 µl of nuclease-free water. This RNA (5 µl) was used as the template in a one-step RT-PCR (Ready-To-Go™ RT-PCR Beads; Amersham Pharmacia Biotech). The following thermal profile was used: 42 °C for 30 min, 94 °C for 5 min, 35 cycles of 94 °C for 60 sec, 55 °C for 60 sec and 72 °C for 90 sec, followed by a final extension of 72 °C for 5 min. PCR products were analysed on a 1.5% agarose-TBE gel containing 0.5 µg/ml ethidium bromide. DNA weight markers (GeneRuler 100 bp DNA Ladder Plus, Ready-To-Use; MBI Fermentas) were run alongside the samples to facilitate product identification and quantification. Post-PCR removal of dNTPs and primers was achieved enzymatically using ExoSAP-IT (GE Healthcare) according to the manufacturer's instructions.

Sequence determination

PCR amplicons were sequenced using the DTS Quick Start Kit (Beckman Coulter) according to the manufacturer's instructions and with the sequencing primers listed in Table 1. The sequencing reactions were run on a CEQ8000™ Automated Sequencer (Beckman Coulter) according to the manufacturer's instructions.

Phylogenetic analysis

A mid-point rooted Neighbor-joining tree was constructed using MEGA version 3.1 (Kumar *et al.*, 2004). The robustness of the tree topology was assessed with 1000 bootstrap replicates as implemented in the program.

Table 1. Primers used for RT-PCR and DNA sequencing.

Primer name	Primer sequence (5' to 3')	Location	Use
C-1C536F	TACAGGGATGGGTCTGTGTGTACC	VP3 (536-559)	PCR
C-1C616F	AAAGACTTTGAGCTCCGGCTACC	VP3 (616-638)	PCR+SEQ
NK61	GACATGTCCTCCTGCATCTG	2B (58-77)	RT+PCR
EUR-2B52R	GACATGTCCTCCTGCATCTGGTTGAT	2B (52-77)	RT+PCR
C-1D535R	ARAGYTCIGCICGYTTCAT	VP1 (535-554)	SEQ
NK72	GAAGGGCCCAGGGTTGGACTC	2A/2B (34-48; 1-6)	SEQ

RESULTS

The primer sets used were able to amplify the VP1-coding region of all the FMDV C isolates examined. The complete VP1 sequences which were determined are shown in red in Fig. 1.

DISCUSSION

Vaccination and other control measures have successfully eradicated FMDV C from Europe and (probably) South America. However, the reason for the disappearance of this serotype from other regions is unclear.

This study probably raises more questions than it answers due to the obvious large gaps in the sample collection.

Could FMDV type C have evolved in Europe and only later spread to Africa, Asia and South America?

Is FMDV C extinct in the field? Or could there be a reservoir somewhere?

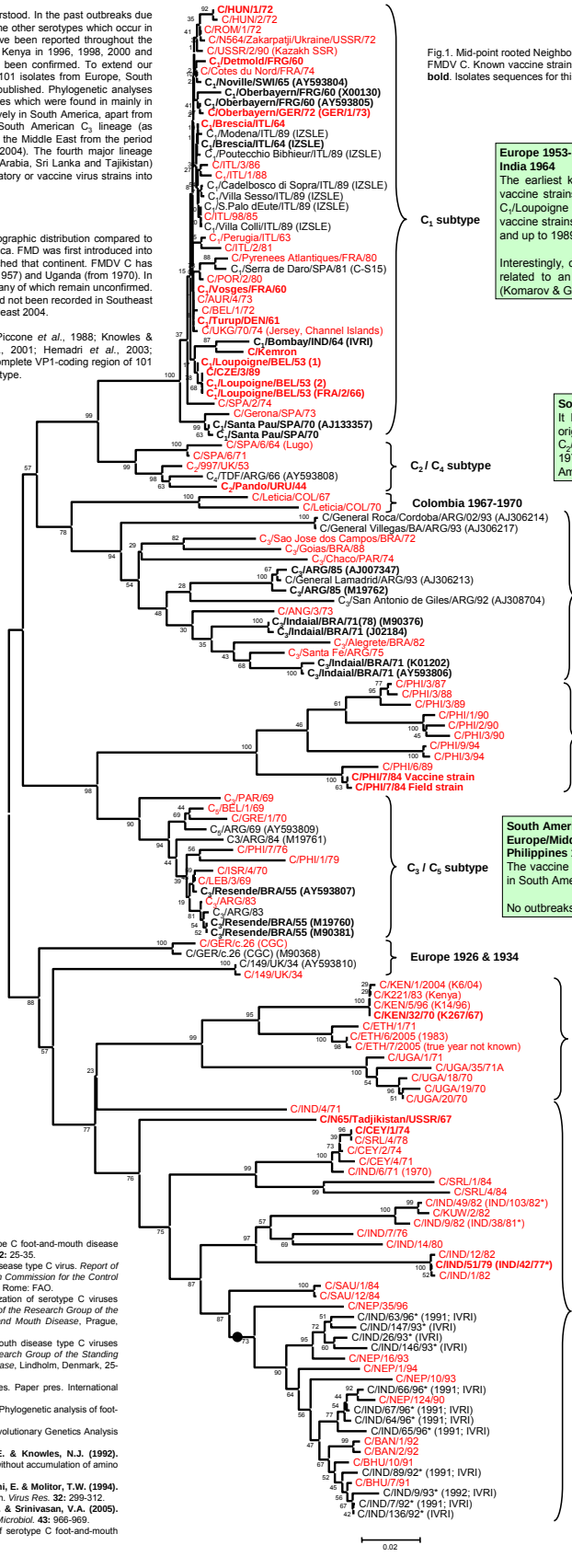

Fig.1. Mid-point rooted Neighbor-joining tree showing the relationships between 146 complete VP1 sequences of FMDV C. Known vaccine strains or field isolates that have later been used as vaccine strains are highlighted in **bold**. Isolates sequences for this study are shown in red. IVRI, Hemadri *et al.* (2003); IZSLE, Meyer *et al.* (1994).

Europe 1953-1989
India 1964
The earliest known isolate belonging to this group is C_1/Loupoigne/Belgium/53. Many European vaccine strains are based on isolates made during the 1960's, all of which are closely related to C_1/Loupoigne (e.g. C_1/Oberbayern, C_1/Noville, C_1/Brescia, C_1/Detmold, C_1/Santa Pau). These vaccine strains have probably been responsible for most European type C outbreaks since that time and up to 1989. No outbreaks due to this lineage have been confirmed since that time.

Interestingly, one of the two non-European isolates in this group, C_1/Bombay/India/64, was closely related to an egg-attenuated vaccine strain produced in Israel in the late 1950's, C Kemron (Komarov & Goldsmit, 1959).

South America 1944 & 1966 / Europe 1953-1964
It has always been assumed that the type C virus introduced into the UK in 1953 originated in South America due to it's relationship with the vaccine strain C_2/Pando/Uruguay/44. However, we have now found C_2-like viruses in Spain in 1964 and 1971. To our knowledge C_2-like viruses have not been reported in the field in South America – perhaps this is a lineage of European origin pre-dating the C_1 lineage?

South America 1971-1993
Angola 1973
Two South American vaccine strains appear in this group (C_3/Indaial/Brazil/71 and C_3/ARG/85). The former appears as two slightly different lineages and it is not clear why. The latter appears to have responsible for one disease outbreak in Argentina in 1993.

The only type C outbreak to have occurred in South America since 1993 was in Careiro de Várzea, Amazonas Region, Brazil in 2004. It was antigenically typed as C_3 and genetically appears to lie mid-way between the two different C_3 groups (I. Bergmann, per. comm. 2004). The sequence was not available for comparison here.

Philippines 1984-1994
These viruses evolved from earlier type C's in the Philippines as evidences amino acid changes in VP1 and 2A. The first is a duplication of an glutamine residue in the B-C loop of VP1 and the second is a serine at position 2 of 2A, both of which are only found in the C_3/C_5 South American lineage.

No outbreaks due to this lineage have been confirmed since 1994.

South America 1955-1983
Europe/Middle East 1969-1970
Philippines 1976-1979
The vaccine strain C_3/Resende/Brazil/55 has been responsible, directly or indirectly, for outbreaks in South America and elsewhere, including the Philippines in 1976 where it became established.

No outbreaks due to this lineage have been confirmed since 1984.

Africa 1967-2004
FMDV C was first recorded in sub-Saharan Africa (Ethiopia & Kenya) in 1957 and remained endemic in Kenya until 1988. In Ethiopia the situation is less clear, but outbreaks were recorded during the 1970's & 1980's. It was first recorded in Uganda in 1970 and was reported sporadically until 1988. The few isolates available from Kenya were all identical to the vaccine strain, K267/67. It can be seen that each East African country for which virus isolates are available show independent lineages with a common origin, possibly indicating a single introduction into this region.

Asia 1967-1996
All virus isolates from the Indian sub-continent are grouped here. Clearly outbreaks have been transmitted to the Middle East on a number of independent occasions, e.g. Kuwait in 1982, Saudi Arabia in 1984.

The 12 partial VP1 sequences of FMDV C viruses from India (isolated between 1977 and 1994) reported by Nagendrakumar *et al.* (2005) all fall within the lineage indicated by the solid circle.

No outbreaks due to this lineage have been confirmed since 1996.

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