

# Molecular Epidemiology of An Asian Pandemic Strain of Foot-and-Mouth Disease Type O Virus



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## INTRODUCTION

The OIE/FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) is established within the high security laboratory at the Institute for Animal Health, Pirbright, U.K. Annually, over 400 samples are received for diagnosis of foot-and-mouth disease (FMD) from throughout the FMD endemic region, which is predominantly Africa, Asia and parts of South America. There are seven serotypes of FMD virus; SAT 1, SAT 2 and SAT 3 restricted to Africa, Asia 1 restricted to Asia and O, A and C present in Africa, Asia and South America and occasionally Europe. In each of the last five years, serotype O has been isolated from over 60% of the positive FMD samples received.

Foot-and-mouth disease virus (FMDV) is a member of the genus *Aphthovirus* within the family *Picornoviridae*, and has a genome consisting of a single strand of positive sense RNA. The virus has a relatively high mutation rate, potentially changing, on a random basis, between one and eight nucleotides per replication cycle. This tendency towards genetic variability has been utilized to individually characterize strains of FMD virus, and track their movement across international borders. A region of approximately 200 nucleotides of the 1D gene, which codes for the major structural protein VP1, was selected for genomic comparison between strains of FMD virus. Sequence differences between two isolates of less than 4% indicates a recent common origin, whereas differences of 15% or more signify geographical isolation over many years. Isolates with greater than 85% nucleotide sequence identity have been placed within groups or topotypes, which tend to be restricted in their geographical distribution.

FMD causes an acute disease of pigs and wild and domestic ruminants such as cattle, water buffalo, sheep, goats and deer. It can cause high mortality in young animals and production losses in adults, and is considered the single most important constraint to trade in live animals and animal products. Movement of FMDV is predominantly associated with the legal and illegal movement of infected animals, although it can spread in animal products, and, under the right conditions, up to 250 km as an aerosol.

We describe here a strain of serotype O FMD virus which we have identified as spreading from India westward into Saudi Arabia and then throughout the Middle East and into Europe, and eastward through Nepal and Bhutan to mainland China, Taiwan Province of China (POC), Japan and South Korea.

## MATERIALS AND METHODS

Total RNA was extracted either directly from epithelial suspensions or from cell culture grown virus using a commercial kit (RNeasy, Qiagen) and an RT-PCR was performed as described by Knowles and Samuel (1994) using primers ARS4 and NK61. The PCR amplicons were partially sequenced using a commercial kit (fmol, Promega) and an automated sequencer (ALFexpress II, Pharmacia) with Cy5-labelled primers NK72, 1D296F, 1C564F and ARS4. UPGMA trees were constructed using the PHYLIP package (Felsenstein, 1993). All these techniques are fully described in a laboratory manual (Knowles and Samuel, 1998) which is downloadable from our website:

<http://www.iah.bbsrc.ac.uk/virus/Picornoviridae/Aphthovirus/fmdv.htm>

Fig. 2. Phylogenetic tree showing the relationships between partial VP1 sequences of viruses belonging to the PanAsian lineage with other FMD type O viruses.

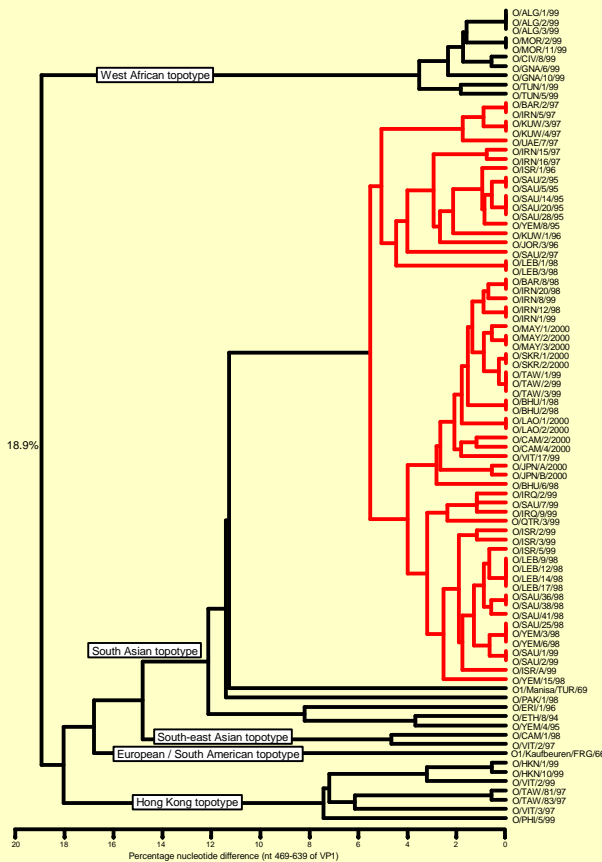
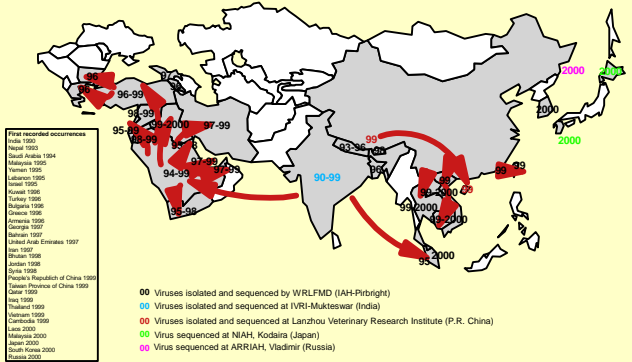


Fig. 1. Conjectured spread of the India-90 (PanAsia) lineage of the South Asian topotype of FMDV-O



## RESULTS & DISCUSSION

The pandemic virus was first isolated from outbreaks of FMD in northern India in 1990. It spread into Saudi Arabia, probably with the live trade in sheep and goats. Saudi Arabia imports over 6 million live animals annually to support the requirements of the Muslim festivals. During 1994 the virus spread within Saudi Arabia and then moved into neighbouring countries so that by 1996 it had reached Turkey. From Turkey it crossed the Euxus river, probably with illegally moved sheep, into Greece, where it caused 39 reported outbreaks. The same virus also caused an outbreak in Bulgaria. During the following two years major outbreaks of FMD were reported from Iran, Iraq, Syria, Israel, Lebanon, Jordan and the Arabian Peninsula all due to the same strain of type O FMD virus (Fig. 1).

Figures 2 and 3 show phylogenetic trees (UPGMA) comparing either partial or complete VP1 sequences, respectively. For purposes of clarity Figure 2 only shows a proportion of the virus isolates that we have identified as belonging to the PanAsian lineage.

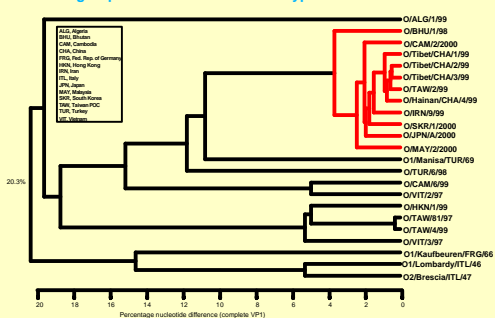
The success of this virus can be measured by the observation that it has now replaced all other strains circulating in the Middle East. It not only caused large-scale disease when it first arrived, but it has persisted, and in 1999 it was responsible for outbreaks of FMD on at least three of the large Saudi Arabian dairy farms. The cattle on these farms are regularly vaccinated with well produced vaccines which laboratory tests indicate should protect the cattle. In spite of vaccination and high security surrounding the farms, the virus was able to gain entry and cause clinical disease.

The eastward spread of the strain is less certain. In 1993 and 1994 it was recovered from samples submitted from Nepal and in 1998 from Bhutan. During 1998 and 1999 there have been outbreaks of FMD in China (Yunnan Province in July 1998; Tibet, Hainan Province and Fujian Province in May 1999). Sequence analysis of three viruses from Tibet (O/CHA/1/99, O/CHA/2/99 and O/CHA/3/99) and one from Hainan (O/CHA/4/99) shows that these outbreaks were caused by the same virus. The first outbreak in Taiwan was on an island (Kinmen) belonging to Taiwan within a kilometre of the Chinese mainland. Cattle were moved from this island to the main island of Taiwan before FMD had been suspected, and initially infection was detected in Tainan prefecture. In January 2000 cattle were affected in Yunlin and Chiayi prefectures and in February 2000, goats were affected in Kaoshiung and Changhua prefectures. In March 2000, FMD type O appeared in South Korea and Japan and sequence analysis revealed the PanAsian strain to be responsible.

Towards the end of 1999 it became clear that the PanAsian virus was moving into South East Asia, where a different FMDV type O topotype normally exists. By April 2000 all mainland South East Asian countries, with the exception of Myanmar, had outbreaks due to the new strain. It has recently been reported that a severe outbreak of FMD type O in pigs in the Ussuriysk district of eastern Russia, was also caused by this new strain (report to OIE).

The extent of this spread is unique for one strain of FMD virus, and its presence in all recent samples from the Middle East indicates that it has out-competed the other strains and topotypes of FMD virus previously established in these areas. Its appearance in South Korea and Japan, previously free of the disease since 1934 and 1908, respectively shows that this strain is capable of spreading to countries where strict measures are undertaken to exclude importation of animal diseases. Whether this fitness to survive is related to its ability to cause more severe clinical disease, as has been indicated from reports from the Middle East, or an increased ability to transmit between susceptible species, whether cattle, sheep or pigs, is not clear. However, its spread across most of Asia and into Europe demonstrates how a newly evolved virus with a competitive advantage can become established, in spite of controls at international borders to prevent the spread of animal disease. The presence of FMD in a previously free country can seriously interfere with the local and export trade in susceptible animals and their products. The appearance in Taiwan Province of China in 1997 of a pig adapted strain of FMD virus has cost the country over 1.5 billion dollars annually in lost export markets, apart from the expenditure on the control programme. A large outbreak of FMD in northern Europe or USA would result in losses exceeding 100 billion dollars. It is not surprising that many nations view with anxiety the threat of economic terrorism based on the use of rapidly spreading biological agents targeted at farm animal. The emergence of this competitively advantaged strain of FMD virus, and its spread within the territory bounded by Greece in the West and Japan in the east, provides an example of the economic damage that can result.

Fig. 3. Phylogenetic tree showing the relationships between the complete VP1-coding sequences of some of the FMD type O viruses studied.



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