

Further Studies on the Molecular Epidemiology and Evolution of Swine Vesicular Disease Virus



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ABSTRACT

The complete VP1 sequences of 48 swine vesicular disease (SVD) viruses were determined by RT-PCR and cycle sequencing, viz. five viruses from Eastern Europe isolated between 1971 and 1987, 12 viruses from the United Kingdom isolated between 1973 and 1982, one virus from Italy isolated in 1977, two viruses from Portugal isolated in 1995, 18 viruses from Italy isolated in 1998 and 1999, five viruses from Hong Kong isolated between 1989 and 1991, and five viruses from Taiwan Province of China (POC) isolated in 1997 and 1998. Examination of the genetic relationships between the 12 SVD viruses isolated in the United Kingdom and a virus from the first UK SVD outbreak (UKG/27/72) revealed a steady evolutionary rate consistent with that found in a previous study (Zhang et al., 1999), approximately 2.5×10^{-3} substitutions per site per year. Phylogenetic analysis of the complete VP1 sequences of seven SVD viruses isolated from pigs in Hong Kong between 1989 and 1991 and five from Taiwan POC in 1997 and 1998 showed that they were most closely related to viruses first isolated in the Netherlands and Italy in 1992. All the viruses from Portugal in 1995 and Italy in 1998 and 1999 were very closely related to the Netherlands/Italy 1992 group, as were five virus isolates from 1996 and seven from 1997 for which partial VP1 sequence data was obtained. Two of the SVD viruses isolated from Eastern Europe (Poland/1/73 and Romania/1/73) and Italy/5/77 were closely related to Hong Kong and Western European viruses prevalent between 1972 and 1979. Bulgaria/2/71 and Odessa/USSR/5/72 were related to a virus found in the Bordeaux region of France in 1973 (FRA/1/73) and to a group of viruses prevalent in Hong Kong between 1971 and 1974. Analysis of this new data has helped establish a more complete understanding of the epidemiology of SVD and supports our hypothesis that the recent Netherlands/Italy 1992 lineage originated in the Far East.

INTRODUCTION

It has been previously shown (Brocchi et al., 1997) that swine vesicular disease (SVD) viruses isolated from outbreaks in Western European countries (Italy, Netherlands and Spain) between the summer of 1992 and 1994 all belonged to a genetic lineage which differed considerably from SVD viruses present prior to 1992. Partial sequencing of an SVD virus isolated in Romania in 1987 was also found to be related to the newly introduced lineage and it was suggested that the origin of the introduction was Eastern Europe (Brocchi et al., 1997). We have now examined, by RT-PCR and nucleotide sequencing, five SVDV isolates from Hong Kong (1989 to 1991), five from Taiwan Province of China (1997 and 1998), 12 from Italy (1995 to 1997) and two from Portugal (1995). Additionally we have sequenced the VP1 gene of a number of older isolates from the United Kingdom and Eastern Europe.

MATERIALS AND METHODS

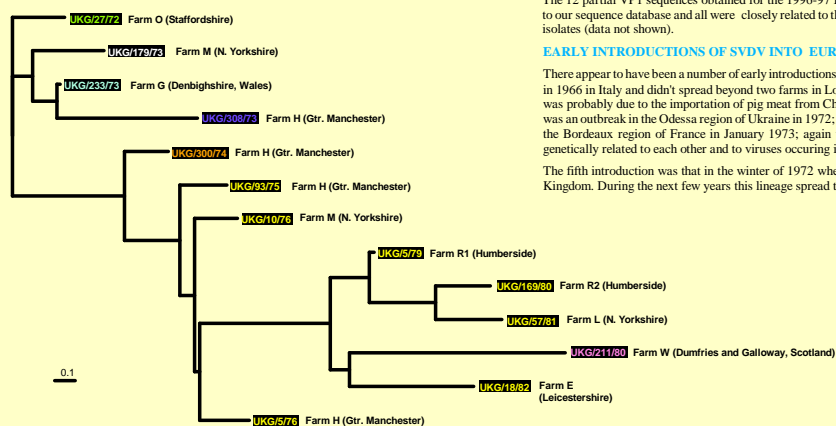
Nucleotide sequence determination

Viruses were grown in tissue culture flasks containing approximately 5×10^6 IB-RS-2 cells. Total RNA was extracted from infected cells and first strand cDNA synthesis was carried out as described previously (Zhang et al., 1993) using the oligonucleotide GSVD-1 (see Brocchi et al., 1997) in a final reaction volume of 20 μ l. The product was extracted with phenol, precipitated with ethanol and resuspended in 15 μ l of water. A 5 μ l sample was amplified by PCR with Taq polymerase (Boehringer-Mannheim, Germany) and 200 nM oligonucleotides (GSVD-3 and NK44; see Brocchi et al., 1997; Fig. 1). 20 μ M deoxynucleotides and buffer as supplied by the manufacturer, in a reaction volume of 50 μ l. Cycling conditions were: 95°C, 4 min; 50°C, 90s; 72°C, 90s; followed by 25 cycles of 94°C, 60s; 50°C, 60s; and 72°C, 60s. An aliquot of the product was analysed by electrophoresis through an agarose gel. The remainder was purified by adsorption to and elution from a silica matrix (Magic PCR prepTM, Promega, WI). Sequencing was done essentially as described in the fmoTM sequencing kit (Promega, WI) using approximately 100 ng of template DNA and oligonucleotides labelled at the 5' terminus with ³²P-ATP (see Brocchi et al., 1997).

Phylogenetic analysis

Nucleotide sequences were analysed on an IBM compatible personal computer using programs written by one of the authors (NJK). All pairwise comparisons were performed by giving each base substitution equal statistical weight (ambiguities were ignored). A binary tree was constructed according to sequence relatedness (either on complete or partial VP1 sequences) using the Neighbor-joining method as implemented in the computer program NEIGHBOR and a dendrogram plotted using the program DRAWGRAM both from the PHYLIP version 3.5c phylogeny package (Felsenstein, 1993).

Fig. 3. Neighbor-joining tree showing the evolution of SVDV in the United Kingdom between 1972 and 1982. The colours indicated distinct SDS-PAGE polypeptide patterns.



EVOLUTION OF SVDV IN THE UNITED KINGDOM, 1972-1982

Following the introduction of SVD into the UK in December 1972, the disease spread rapidly peaking with 187 outbreaks in 1974. SDS-PAGE analysis demonstrated differences between some of the UK SVD viruses during its 10 year presence (Harris et al., 1975, 1979; N.J. Knowles, unpub. data). Analysis of the complete VP1 sequences of 13 SVD viruses isolated in the UK between 1972 and 1982 indicate a clock-like evolution without the introduction of new strains (Figs. 3 and 4). The rate of fixation of mutations was calculated to be 2.45×10^{-3} substitutions/site/year, consistent with previous estimates (Zhang et al., 1998). The re-occurrence of SVD on some farms (Fig. 3) following control measures (slaughter of pigs and disinfection of the premises) would appear to be the result of new introductions of virus rather than re-infection with residual virus from the previous outbreak.

ACKNOWLEDGEMENTS

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Fig. 2. Neighbor-joining tree showing the relationships between the complete VP1 sequences of the SVD viruses studied. The actual year of collection is in parentheses if different from reference number

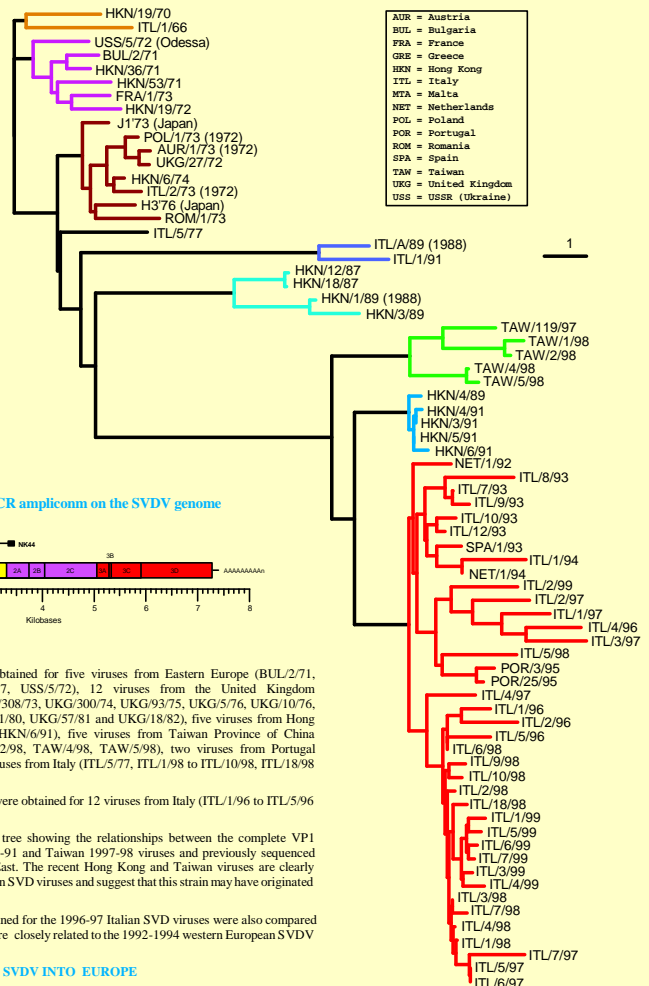
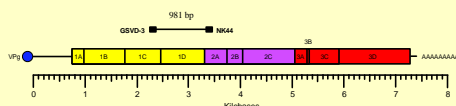


Fig. 1. Location of RT-PCR amplicon on the SVDV genome



RESULTS AND DISCUSSION

Complete VP1 sequences were obtained for five viruses from Eastern Europe (BUL/2/71, POL/1/73, ROM/1/73, ROM/1/87, USS/5/72), 12 viruses from the United Kingdom (UKG/179/73, UKG/233/73, UKG/308/73, UKG/300/74, UKG/93/75, UKG/5/76, UKG/10/76, UKG/5/79, UKG/169/80, UKG/211/80, UKG/57/81 and UKG/18/82), five viruses from Hong Kong (HKN/4/89, HKN/3/91 to HKN/6/91), five viruses from Taiwan Province of China (TAW/119/97, TAW/1/98, TAW/2/98, TAW/4/98, TAW/5/98), two viruses from Portugal (POR/3/95, POR/25/95) and 19 viruses from Italy (ITL/5/77, ITL/1/96 to ITL/10/98, ITL/18/98 and ITL/1/99 to ITL/7/97).

Partial VP1 sequences (3' 300 nt) were obtained for 12 viruses from Italy (ITL/1/96 to ITL/5/96 and ITL/1/97 to ITL/7/97).

Fig. 2 depicts a Neighbor-joining tree showing the relationships between the complete VP1 sequences of the Hong Kong 1989-91 and Taiwan 1997-98 viruses and previously sequenced viruses from Europe and the Far East. The recent Hong Kong and Taiwan viruses are clearly related to the 1992 western European SVD viruses and suggest that this strain may have originated in China.

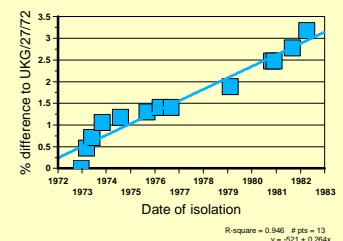
The 12 partial VP1 sequences obtained for the 1996-97 Italian SVD viruses were also compared to our sequence database and all were closely related to the 1992-1994 western European SVDV isolates (data not shown).

EARLY INTRODUCTIONS OF SVDV INTO EUROPE

There appear to have been a number of early introductions of SVDV into Europe from the Far East. The first occurred in 1966 in Italy and didn't spread beyond two farms in Lombardy. The second was in Plovdiv, Bulgaria in 1971 and was probably due to the importation of pig meat from China (Y. Ivanov, personal communication, 1993). The third was an outbreak in the Odessa region of Ukraine in 1972; the origin of this outbreak is not known. The fourth was in the Bordeaux region of France in January 1973; again the origin is unknown. The latter three outbreaks are all genetically related to each other and to viruses occurring in Hong Kong in 1971-73 (Fig. 1; Zhang et al., 1998).

The fifth introduction was that in the winter of 1972 when SVD appeared in Poland, Austria, Italy and the United Kingdom. During the next few years this lineage spread throughout most of western Europe.

Fig. 4. Time vs distance plot of the SVDV isolates from the United Kingdom



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