

Re-emergence of Encephalomyocarditis Virus in Italy: Antigenic and Molecular Characterization of Viruses Isolated From Pigs with Acute Fatal Myocarditis Between 1996 and 1999

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ABSTRACT

After an absence of nearly ten years, fatal myocarditis in pigs, due to encephalomyocarditis virus (EMCV), reappeared in Italy in 1996. A severe, but geographically isolated case, occurred in a breeding herd in north-eastern Italy in October 1996. Following a gap of one year EMCV then appeared in southern Lombardy. The disease remained endemic in a small area between November 1997 and October 1999, resulting in a total of 30 outbreaks (Fig 1). Two further outbreaks also occurred on farms which were connected to the episode in 1996. Serological (ELISA) analysis performed with 40 monoclonal antibodies (MAbs) raised against the Italy/86 strain, showed no significant antigenic variation in EMCV isolates from 1986 to 1996. All the isolates from 1997 to 1999 had a reduced reactivity with the neutralising MAb 4H2 which recognizes a conformational epitope on the virus capsid. With a few exceptions, other epitopes were well conserved. Part of the capsid-coding region of most of the new virus isolates was amplified by RT-PCR and the complete VP1 sequences determined. These were compared with sequences previously determined for EMC viruses isolated in Europe since 1986 and a phylogenetic tree was constructed.

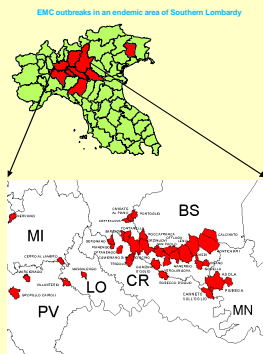


Figure 1.

MATERIALS AND METHODS

Virus Isolation: Tissue homogenates were inoculated onto BHK-21 cell monolayers, inspected daily for CPE and then passaged twice (on BHK-21 cells).

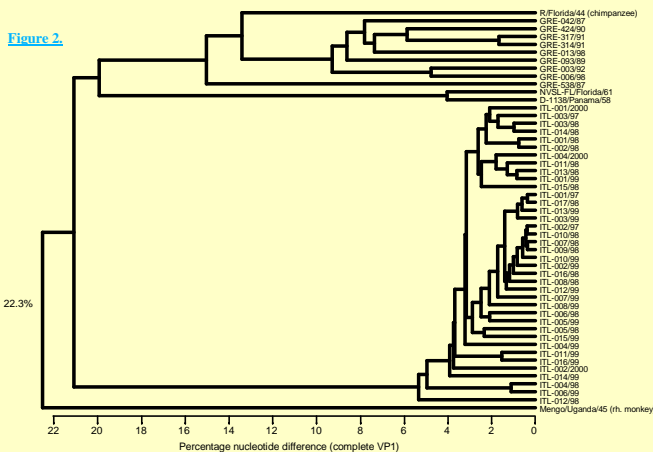
Antigen detection: A MAbs-based sandwich ELISA (Brocchi et al., 1998) was used to identify EMCV, either directly in tissue homogenates, or in inoculated cultures showing CPE.

Antigenic characterisation: A panel of 40 MAbs were used in an antigen trapping ELISA as previously described (Koenen et al., 1997).

RNA preparation/RT-PCR/sequence determination: These protocols were used as described previously (Knowles et al., 1998; Koenen et al., 1999). Oligonucleotide primers were designed based on published sequences of EMCV strains; R, Mengo, B, D and PV21 (EMBL/GenBank/DBJ accession numbers M81861, L22089, M22457, M22458 and X74312, respectively) and synthesised by commercial companies (Cruachem, UK and Amersham Pharmacia Biotech, UK).

Phylogenetic analysis: Nucleotide sequences were analysed on an IBM compatible computer using programs written by one of the authors (NJK). All pairwise comparisons were performed by giving each base substitution equal statistical weight were ignored. A UPGMA was constructed using the program "NEIGHBOR" - part of the "PHYLIP" phylogenetic reconstruction package (Felsenstein, 1993).

Figure 2.



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Figure 3.

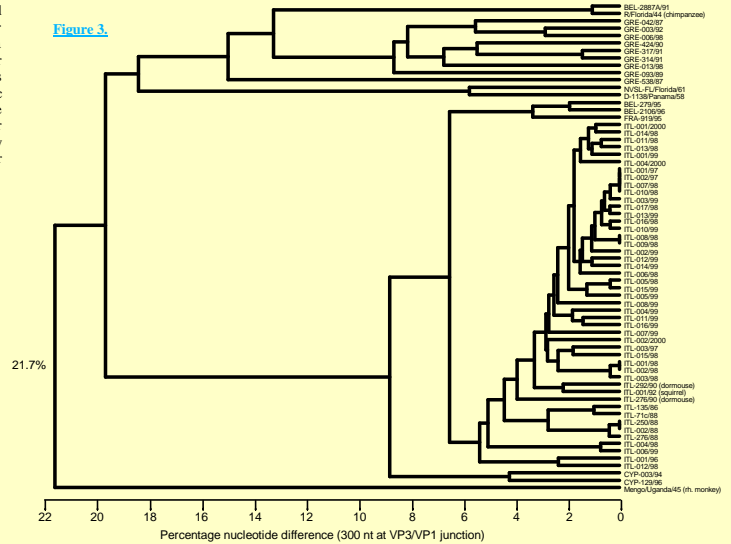


Table 1.

MAb	Neutralising MAbs							Non-neutralising MAbs						
	ext	ext	ext	ext	ext	ext	ext	ext	ext	ext	ext	ext	ext	ext
A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(CONF., C. = conformational, LIN. = linear, ext. = external, int. = internal)

MAB	01/97, 02/97, 01/98, 02/98, 04/98, 07/98, 14/98, 17/98, 08/99, 10/99, 11/99, 14/99, 15/99
A	03/97, 16/98
B	03/98, 05/98, 06/98, 08/98, 09/99
C	05/98, 10/98, 11/98, 12/98, 13/98, 15/98, 01/99, 02/99, 03/99, 04/99, 06/99
D	18/98, 06/99
E	05/99, 07/99
F	05/99, 07/99
G	05/99, 07/99
H	13/99, 01/00
I	16/99, 03/00, 04/00, 05/00, 07/00
L	02/00, 10/00, 11/00

KEY	REACTIVITY
+	100%
+	50%
+	0%

RESULTS AND DISCUSSION

The 1D region of EMCV (coding for capsid protein VP1) displays considerable genetic variability, with over 22% sequence variation between nucleotide sequences of viruses isolated from different hosts in different countries (Fig. 2). However, all the Italian isolates that have been sequenced so far are genetically very closely related and form a discrete lineage that is distinct from EMCV reference strains and also from viruses isolated in Greece. The sequence variation within the VP1 region of the Italian EMCV strains is less than 6%, indicating a high degree of genetic stability within the endemic area over the last 5 years. Even over the last 14 years, nucleotide differences between the Italian viruses at the VP3/VP1 junction have not exceeded this level (Fig. 3).

The Italian EMCV isolates are also very stable antigenically (Table 1). The observed random occurrence of minor variants in MAB reactivity may indicate that the virus exists in the wild as a population of quasispecies from which one strain may emerge to cause disease in pigs. The exact nature of the reservoir host is uncertain, although wild rats and other rodents have frequently been implicated in the transmission and spread of the disease.

There is no correlation between MAB reactivity and the variation in the VP1 nucleotide sequence and neither of these two parameters seem to be correlated with the date of virus isolation. In contrast, several of viruses which show a particularly high degree of genetic relatedness were found to come from the same geographical area, despite a temporal separation of many months or even years. This relationship suggests that many of the outbreaks could be the result of spontaneous emergence from local, genetically distinct virus reservoirs, rather than being entirely attributable to the movement of infected livestock. If so, disease control in endemic areas may be dependent on the identification of the reservoir host and characterisation of the factors involved in inducing the emergence of clinical disease in pigs.

ACKNOWLEDGEMENTS

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