Molecular Epidemiological Analysis of Recent Thai Isolates of Foot and Mouth Disease Virus Type O (2001 and 2002)

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Introduction

Foot and mouth disease (FMD) is recognized as the most fearful animal disease in the world at economical point of view. The causative agent, FMDV has not only seven immunologically distinct serotypes (O, A, C, Asia1, SAT 1, SAT2, SAT3) but also has antigenic variation within each serotype. According to our continuous study of typing of Thai isolates, type A and type O have been circulating in recent years with the latter type being predominant (unpublished data). A recent molecular epidemiological work concerning FMDV type O (isolates from '92 to 99, total 5 isolates) showed that Thai isolates belong to South East Asian (SEA) topotype with some variation (1). Our objective was to reveal the most recent molecular epidemiological features of FMDV type O in Thailand.

Materials and Methods

Viruses: All the field specimens were summarized in Table 1. Ten percent suspensions were inoculated to BHK or primary lamb kidney cells to isolate viruses. Antigen capture ELISA was performed following standard protocol (2) for typing.

RNA: Total RNA was extracted from infected cells or infected culture fluid using Trizol (Gibco BRL).

RT-PCR: Ready-to-Go RT-PCR Kit (Pharmacia) was used with O-1C609 and NK61 primer set (1) (3).

Sequencing: Manual sequencing was performed using NK72 primer with PCR Product Sequencing kit (USB). To confirm the sequences, automatic sequencing was also performed using NK61 and 1D296F primers (1) by ABI 310 genetic analyzer.

Sequence Analysis: GENETYX software was used for arrangement of sequences.

Phylogenetic analysis: the Neighbor-joining method implemented in the PHYLIP package (5) was used with FMDV sequence database at IAH Pirbright.

Table 1: Origin of FMD type O recent isolates in Thailand examined in this study

Virus designation	Province	Region	Species	Date collected
F539/44R1B6	Uttaradit	N		2001
F2344R1B4	Phrae	N		2001
51-2/02B4	Udornthani	NE	Pig	2002
44-2/02R2B3	Kalasin 2	NE	Buffalo	2002
59/02B5	Nakhorn-pathom	W	Pig	2002
56-4/02B5	Ratchaburi	W	Pig	2002
72-2/01	Nakorn Si- Thamarat	S	Pig	2001
92/01	Surat Thani	S	Buffalo	2001
$O_{1\,89}$ /Udorn/87*	Udornthani	NE	Cattle	1987

* Thai type O vaccine strain

Results and Discussion

RT-PCR: Total RNA extracted from infected cell was subject to RT-PCR. All of the isolates except isolate, 92/01, were amplified using the same primer as Materials & Methods. For the 92/01 isolate, 1DROD1 (nt from 43 to 62 of FMDV VP1) was used as forward primer with the standard protocol (3). The RT-PCR products were subject to agarose gel electrophoresis to check the size and amount of authentic products (Data not shown).

Sequencing: RT-PCR products were directly labeled with ^{35}S dATP then applied to 4% acryl amide sequence gel. Agarose gel purified RT-PCR products were also labeled with BIG-Dye terminator labeling kit (ABI) and subjected to auto-sequencing. Both sequences from manual and auto-sequencing were compared each other and confirmed the sequence. The corresponding region on each isolate sequence to nucleotides (nt) from 469 to 639 of FMDV O1/Kaufbeuen VP1 sequence (X00871) (4) was determined by homology alignment (data not shown).

Phylogenetic Analysis: The nt 469 to 639 sequences of VP1 were subjected to phylogenetic analysis comparing to those of isolates of neighboring countries (Fig 2).

The phylogenetic tree suggests that recent Thai FMDV type O isolates fall into one topotype (SEA - South East Asia), but belong to a variety of strains/lineages, i.e. Mya98, Cam94 & another unnamed. In the tree shown viruses belonging to the SEA topotype appear as two independent lineages, however, this branching may not be reliable due either to the short length of the sequences used or to the almost exclusive use of recent virus isolates which may be more distantly related to each other than their ancestors.

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References

1. Samuel and Knowles, 2001. JGV 82 : 601-621
2. Manual of Standards for Diagnostic Tests and Vaccine, 2000. OIE.
3. Knowles and Samuel, 1998. RT-PCR and Sequencing Protocols for the Molecular Epidemiology of Exotic Virus Diseases of Animals. Pirbright : Institute for Animal Health 37.
4. Forss et al,. 1984. Nucleic Acids Res 12 : 6587-6601.
5. Felsenstein, 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distrib. by the author. Dept. of Genetics, Univ. of Washington, Seattle.

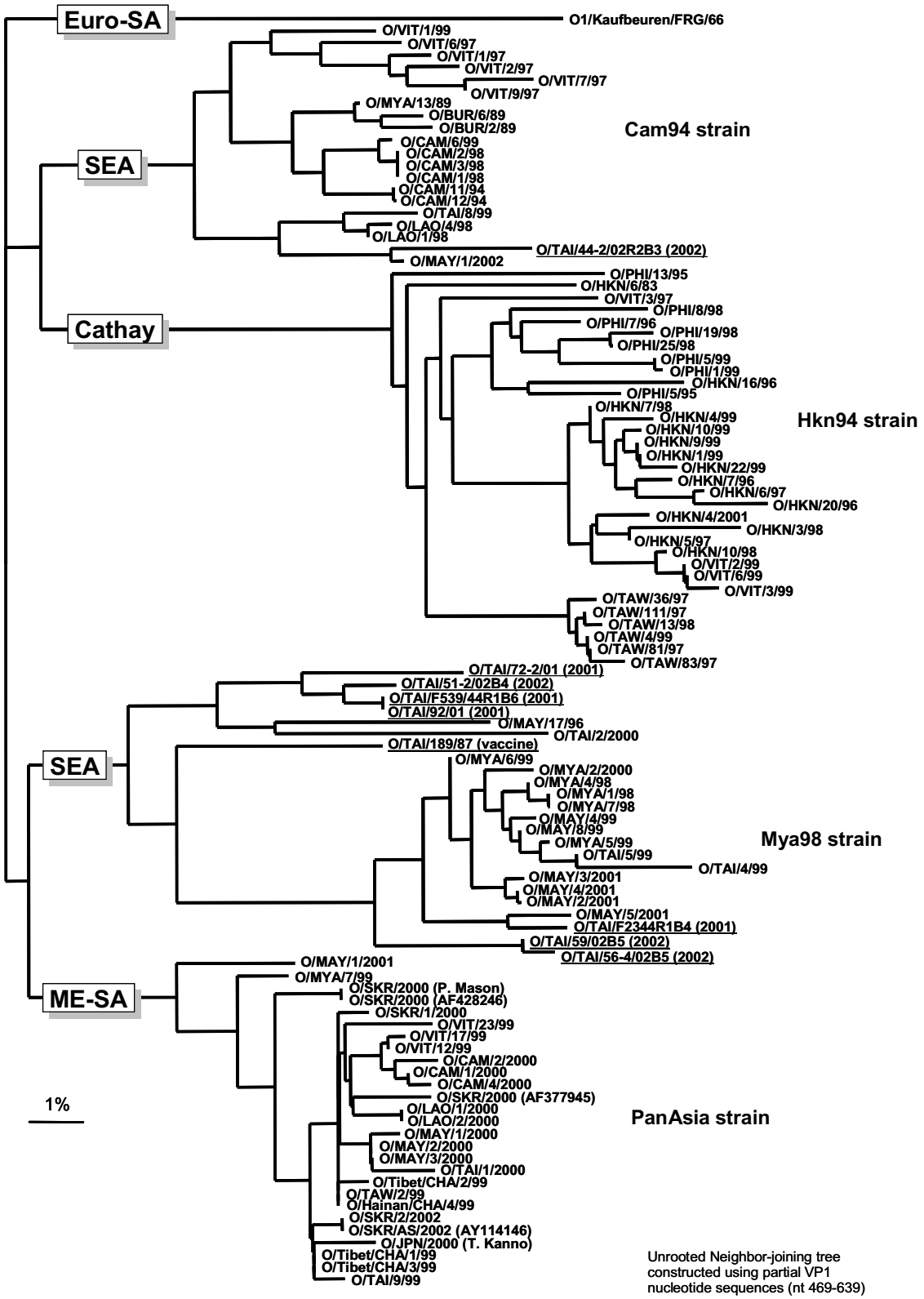

Fig. 1: Phylogenetic tree of recent Thai isolates with Thai vaccine starain of FMDV Type O (underlined).