Molecular Epidemiology Surveillance of Foot and Mouth Disease Virus type A in Thailand during 2012-2014

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Introduction: Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic impact. The disease affects cattle and swine as well as sheep, goats and other cloven-hoofed ruminants. The organism which causes FMD is an Aphthovirus of the family Picornaviridae. It consists of a small, non-enveloped capsid and a single strand of positive sense RNA. There are seven serotypes of the virus O, A, Asia1, C, SAT1, SAT2 and SAT3. Infection or vaccination with one serotype does not confer protection against other serotypes (Mattion et al., 2004). In the past few years, FMDV serotype A is considered endemic in Thailand. Therefore in this study, we used the nucleotide sequencing method to investigate the genetic characterization of foot and mouth disease virus serotype A in Thailand during 2012-2014. The molecular epidemiological information in this study is benefit for database, investigation of genetic variables and support the seed virus selection to enhance the efficacy of vaccine production.

Materials and Methods:
Viruses: Twenty seven samples of FMDV serotype A in Thailand during 2012-2014 were conducted by ELISA typing (Roeder and LeBlanc Smith, 1987).
RNA: Total RNA was extracted from epithelial suspension or cell culture by using Trizol® LS reagent (Invitrogen™, USA) and re-suspended in nuclease-free water.
Reverse Transcription (RT): The single-stranded RNA is reverse transcribed into complementary DNA by using a reverse transcriptase enzyme, following thermal profile was used: 42°C for 60 min and 95°C for 5 min. The newly synthesized cDNA was amplified using for the template in the Polymerase Chain Reaction.
Polymerase Chain Reaction (PCR): cDNA was used as the template in the PCR. The following thermal profile was used: 94°C for 4 min; 30 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 by electrophoresis. Post-PCR removal of deoxynucleoside triphosphates and primers were achieved using the QIAquick PCR Purification Kit (QIAGEN, Germany) and resuspended in nuclease-free water. RT-PCR was performed using foot and mouth disease virus (FMDV) specific primers 1C-612 and NK61 (Knowles and Samuel, 1998) targeting regions either side of the VP1 gene (Table 1).
Sequencing: PCR amplicons were sequenced by using the Big-Dye® terminator cycle kit version 3.1 (Applied Biosystems, USA), with the 1C-612 and NK-72 sequencing primers (Table 1). The sequencing reactions were run on an automated sequencer (ABI 3130 Genetic Analyzer).
Phylogenetic analysis: A Neighbor-joining (unrooted trees) was constructed by using MEGA version 4 (Tamura, et al., 2007). The robustness of the tree topology was implemented in the program.

Table 1 Oligonucleotide primers used for RT-PCR and sequencing of FMDV serotype A.

Primer	Primer sequence (5'-3')	Gene	Product length	Use
1C-612	TAGCGCCGGCAAAGACTTTGA	1C	813 bp	RT-PCR, Sequencing
NK72	GAAGGGCCCAGGGTTGGACTC	2A/2B	Universal primer	Sequencing
NK61	GACATGTCCTCCTGCATCTG	2B	Universal primer	RT-PCR

Results: Twenty seven samples of foot and mouth disease virus serotype A in Thailand during 2012-2014 were performed by ELISA typing and PCR technique using specific primer 1C-612/NK61. The PCR product (813 bp) analysis was performed using nucleotide sequencing. The complete sequence of 636 nucleotides VP1 genome from isolates of serotype A were compared and analyzed as phylogenetic tree. It was found that all of FMDV serotype A did not have genetic variables and belonged to ASIA topotype (Fig 1).

Conclusions: Foot and mouth disease virus serotype A in Thailand during 2012-2014 did not have genetic variable and belonged to ASIA topotype. The information in this study can be used to support the seed virus selection to enhance the efficacy of vaccine production.

Keywords: Foot and mouth disease virus, serotype A, nucleotide sequencing, topotype

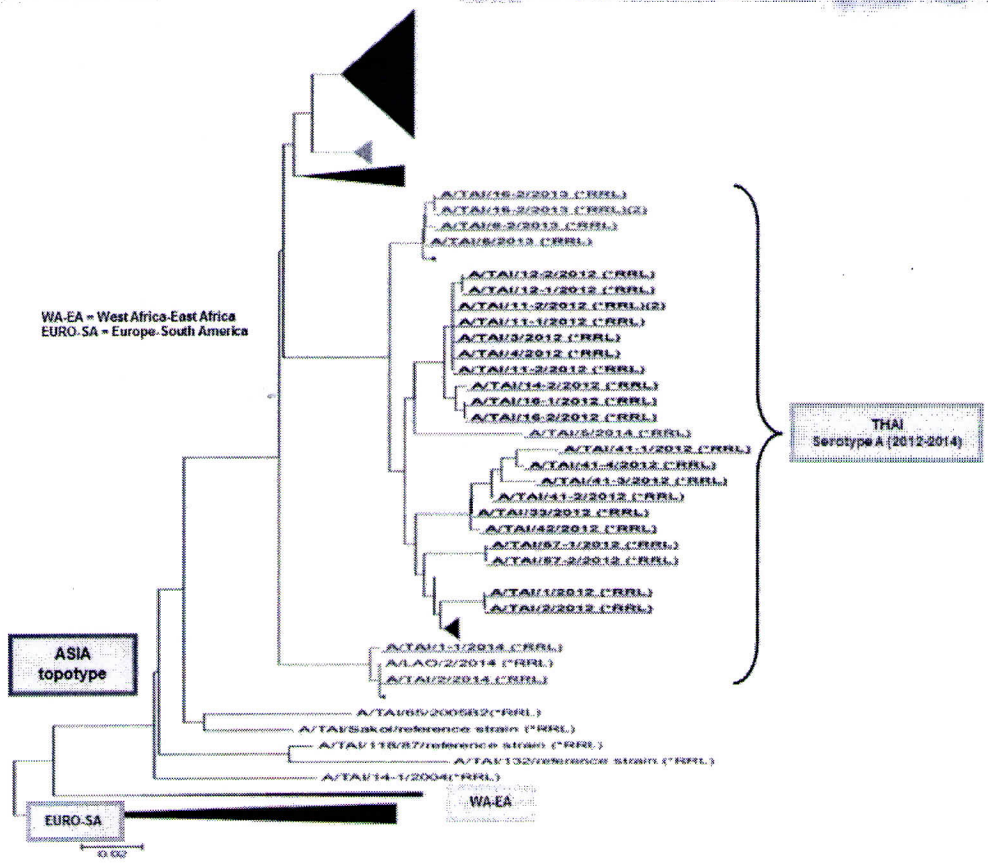

Fig 1 Phylogenetic tree of FMDV serotype A in Thailand during 2012-2014 (underline)

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