Vaccine matching strain characterization of foot and mouth disease virus in South East Asia during 2010-2012

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Abstract

The vaccine matching strain characterization of foot and mouth disease virus type O and A causing outbreak in Thailand, Cambodia, Lao PDR, Vietnam, Myanmar and Sri Lanka during 2010 to 2012 was investigated. The result was expressed as r-value by determining the serological relationship between field virus isolate and reference virus vaccine strain. The r-value of FMDV type O from Thailand, Cambodia, Lao PDR and Vietnam demonstrated very close antigenic relationship to vaccine strains with O189/87. It is therefore suggested that the existing seed vaccine strains for type O need not to be changed. In contrast, the r-value of type A causing outbreak in Thailand demonstrated a close antigenic relationship to A/Sakolnakorn/97 than A118/87 current vaccine strain. This study indicated that recent type A virus gave antigenic diverse from A118/87 vaccine strain, therefore, it was recommended to put an additional of A/Sakolnakorn/97 vaccine in the existing trivalent vaccine production in order to give a wide broader protection of type A outbreak in the field. In conclusion, the recent field outbreak of type O in South East Asia (SEA) indicated that no antigenic variation has been found, while the antigenic variation of type A has been found only in Thailand, due to less FMD sample of type A from SEA country was submitted to the laboratory at that period. However this study would be useful in supporting the selection of appropriate virus vaccine strain for production of high efficacy vaccine and enhancing the efficiency of the strategic planning for FMD control in SEA region.

Background

Foot and mouth disease, a highly contagious disease of cloven-hoofed animals, is important in South East Asia region. Serotypes O, A and Asia1 are considered endemic in Thailand and South East Asia (SEA), causing significant economic losses primarily due to lower production of affected animals and subsequent constrain of international trading. Rapid and accurate diagnosis plays an important role in the prevention of disease spread and can ensure that appropriate vaccines are selected for use against circulating filed strains. Standard ELISA typing test for type identification of field samples (Roeder and Le Blance Smith, 1987) and other serological tests including virus neutralization (VN) test (Rweyemamu,1978), liquid phase blocking ELISA (LP ELISA) (Hamblin et al.,1986) in parallel with ELISA-non structural protein (ELISA-NSP) (Linchongsubongkoch et al., 2008), can be used to support disease surveillance and sero-monitoring of vaccinated and infected animals. In addition, strain differentiation investigation by vaccine matching study of field viruses and reference vaccine

strains become a very important in supporting a scientific information of the antigenic variation in FMD field outbreak strains. The vaccine matching investigation was carried out by determination of the serological relationship (r-value) between filed virus strains and the reference vaccine strains and is useful for selecting the appropriate virus strain for vaccine production and enhancing the vaccination strategic of FMD control at the national and regional level. Linchongsubongkoch et al. (2008) reported that the r-value of type O causing outbreak during 1997-2007 and 2008-2009 (data not published) indicated that the serological relationship was close to O189/87 vaccine strain, while type A showed antigenically changed from time to time , it was indicated that the type A field viruses causing outbreak during 2001-2007 and during 2008-2009 were closely related to A118/87 vaccine strain. Therefore, in this study, a number of field isolate viruses type O and A causing outbreak in SEA region during 2010-2012 were studied for updating the epidemiological information and recent FMD antigenic variation by determining the serological relationship (r-value) between field virus strains and the reference virus vaccine which would be useful to support the selection of appropriate virus strain for vaccine production and control of FMD in SEA region.

Materials and methods

1. Reference viruses and field viruses

Reference viruses vaccine strain O189/87, A118/87 and A/Sakolnakorn/97 were obtained from current seed vaccine strains. Field samples from FMD infected animals submitted for laboratory diagnosis which were from Cambodia, Lao PDR, Myanmar, Vietnam, Sri Lanka and Thailand during 2010-2012 which subjected for serotype identification using standard ELISA typing and the virus isolation test by inoculating to primary lamb kidney cell for 2-3 passages and further 4 or 5 passages in BHK-21 cell line. Then cell culture supernatant fluid was again confirmed by antigen typing test as described by Roeder and Le Blanc Smith (1987). The reference vaccine strain and field isolate viruses were titrated by indirect sandwich ELISA method (Kitching et al., 1988) and selected the working dilution for virus to use in the liquid phase blocking ELISA (LP ELISA) (Linchongsubongkoch et al., 2000).

2. Bovine antisera

The reference sera used in the LP ELISA, including bovine anti FMD type O189/87, A118/87 and A/Sakolnakorn/97 were prepared from experimental cattle that have been vaccinated with reference homologous vaccine strain. Blood from vaccinated animals were taken at 21 days post vaccination for immune sera.

3. Liquid phase blocking ELISA (LP ELISA)

Bovine antiserum against homologous vaccine strain was used to determine antibodies to FMD virus by LP ELISA . The bovine serum was diluted into two fold dilution series, and then a fixed concentration of reference vaccine strain and field isolate viruses giving an optical density (OD) in the range of 1.0 –1.5 were reacted with bovine post vaccination serum with homologous virus of each serotype. The antibody titer to FMD virus was determined as described by Hamblin et al. (1986), Kitching et al.(1988) and Linchongsubongkoch et al. (2000).

4. The serological relationship (r-value)

The LP ELISA method has been used to examine the serological relationship between field isolate viruses and the reference virus vaccine strains which was expressed as r-value.

$$\text{r-value} = \frac{\text{Serum titer against heterologous field strain}}{\text{Serum titer against homologous vaccine strain}}$$

The guideline suggestion for r-value obtained by LP ELISA and criteria of interpretation were described by Samuel et al. (1990) as this follows.

r = 0-0.19 highly significant serological variation from the reference strain

r = 0.20-0.39 significant difference from the reference strain, but protection may be satisfactory if using a sufficiently potent vaccine.

r = 0.40-1.0 not significantly difference from vaccine strain.

Results

FMDV samples from SEA country causing outbreak during 2010-2012 were submitted to the Regional Reference Laboratory, Pakchong, Thailand for FMD diagnosis, the diagnostic results were shown in table 1. Then, some of them were used for further investigation by vaccine matching test, the result of r-value was shown in table 2, 3 and figure 1.

Table 1. Situation of FMD outbreak in SEA during 2010-2012, diagnostic assay using standard ELISA typing test and virus isolation.

Year	Country	No. of sample	Type identification by ELISA typing			
			O	A	Asia1	NVD*
2010	Thailand	48	23	4	-	21
	Cambodia	20	12	-	-	8
	Myanmar	3	-	2	-	1
	Vietnam	27	26	1	-	-
2011	Cambodia	31	16	-	-	15
	Lao PDR	20	16	-	-	4
	Sri Lanka	4	2	-	-	2
	Thailand	64	13	37	-	14
2012 (Feb)	Thailand	4	-	4	-	-
Total		221	108	48	-	65

NVD = no virus detected

Table 2. Result of r-value of FMD type O field viruses in Thailand, Cambodia, Lao PDR and Vietnam during 2010-2012, using O189/87 as a homologous system.

Country	Year	Total sample	% r-value range of type O		
			0-0.19	0.20-0.39	0.40-1.0
Cambodia	2010	3	-	-	3
	2011	2	-	-	2
Vietnam	2010	11	-	-	11
Lao PDR	2011	3	-	-	3
Thailand	2010	7	-	-	7
	2011	13	-	-	13
	2012	*		Not done	
		39			39 (100%)

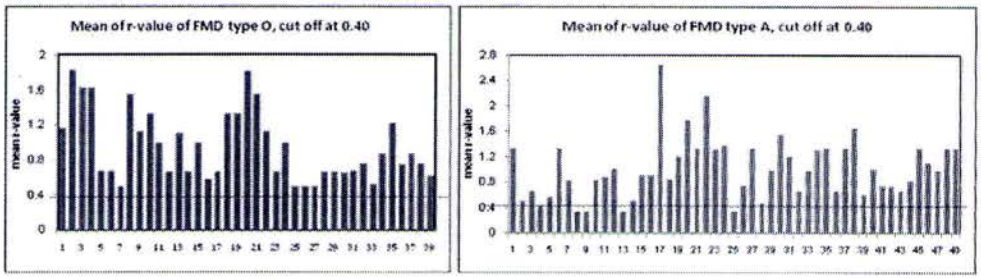
* no sample of type O was received in February,2012

Table 3. Result of r-value of FMDV type A field viruses in Thailand and Myanmar during 2010-2012, using A118/87 and A/ Sakolnakorn/97 as homologous system.

Year/Country	Total sample	r-value range by A118/87			r- value range by A/Sakolnakorn/97		
		0-0.19	0.20-0.39	0.40-1.0	0-0.19	0.20-0.39	0.40-1.0
2010 -Thailand	3	no binding reaction by titration			-	-	3
Vietnam	1	"--------"			-	-	1
Myanmar	2*	Not done			Not done		
2011- Thailand	43	no binding reaction by titration			-	4	39
2012-Thailand	2	no binding reaction by titration			-	-	2
	51					4(8.1%)	45(91.9%)

Remark: *FMD type A of Myanmar was not investigated for r-value, due to no virus could be adapted in cell culture

Figure 1. Result of mean r-value of field isolate virus of type O and A, r-value greater than 0.40 defined as good matching



Conclusion and discussion

The vaccine matching strain characterization or antigenic variation of FMDV in SEA during 2010-2012 was investigated by determining the serological relationship between the reference vaccine viruses and field viruses, the result was expressed as r-value. Table 2 showed that 100% of filed viruses type O from Cambodia, Lao PDR, Vietnam and Thailand gave the r-value greater than 0.40 indicated that the serological relationship was very close to virus vaccine strain O189/87, therefore the current vaccine of O189/87 could give a protection to the circulating viruses in Thailand and the region. While the situation of type A was different, the r-value in table 3 showed the 91.9% of field viruses from Thailand gave the r-value greater that 0.40, and the rest of 8.1% gave the r-value in range 0.20-0.39 to A/Sakolnakorn/97 but not A118/87 system. Similarity result was found in the FMD type A antigenic profiling test demonstrated that no binding reaction to the A118/87 system but distinguished in high binding reaction with A/ Sakolnakorn/97 system (data not published). Therefore, the recent situation of type A field viruses during 2010-2012 have antigenic changed from current vaccine of A/118/87 to A/Sakolnakorn/97 that might be resulting from disease outbreak from time to time (Doughty et al.,1995). By the history of type A seed vaccine selection, A/Sakolnakorn/97 was selected as a new seed vaccine strain and being used from 1997-2001, then again a new seed vaccine strain of A118/87 was selected in 2001 and being used up to the present (Linchongsubongkoch et al., 2008). Interestingly, the vaccine matching result of type A causing outbreak during 2010-2012 was demonstrated the antigenically changed from A118/87 to A/Sakolnakorn/97. In this regard, it was necessary to select a new vaccine strain again, therefore, it was recommended to use the monovalent vaccine of A/Sakolnakorn/97 as an additional vaccine in the existing trivalent vaccine in order to give a wide broader protection to the field outbreak viruses. However,

the nucleotide sequencing of FMD field outbreak was also investigated and analyzed as phylogenetic tree by World Reference Laboratory, (WRL) (data not published). The lineage of FMD type O in SEA region was majority defined as South East Asia (SEA) topotype, Mya98 strain and some was defined as PanAsia strain. It was interesting that FMD sample from Myanmar was submitted to Regional Reference Laboratory at Pakchong, Thailand in October 2010, the initial ELISA typing resulted as type A and unfortunately this virus was not be able to adapted in cell culture for further investigation of vaccine matching test. Hence this viral fluid and RNA sample was sent to World Reference Laboratory for FMD, Pirbright Laboratory, UK for sequencing analysis, the phylogenetic tree indicated that type A from Myanmar/2010 was close related to the virus originally from A/India/2000 (IND/2000) (report from WRL, data not published). However FMD type A in Thailand and Vietnam was defined as only one topotype of ASIA (Asia97 strain). This studied would be useful for tracing back to the original virus causing outbreak in the field and to give a molecular epidemiological information in supporting the seed virus selection for vaccine production in enhancing the action plan for FMD control in the SEA region.

Keywords: FMDV, vaccine matching, r-value, LP ELISA

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