Antigenic evolution of foot and mouth disease virus strains in Thailand

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Abstract

Type identification of foot and mouth disease virus (FMDV) in Thailand was carried out using standard ELISA typing test. The samples collected during the past 11 years were used for evolution study of antigenic variation of FMDV type O, A and Asia1 by liquid phase blocking ELISA (LP ELISA). The results were expressed as the serological relationship (r-value) between the ratio of serum titer against heterologous field strain and homologous vaccine strain. R-values of type O and Asia1 showed close antigenic relationship to vaccine strains (O/189/87 and Asia1/85) respectively. It is therefore suggested that the existing seed vaccine strains for these serotypes need not to be changed. In contrast, the r-value of type A demonstrated antigenic variation compared to that of vaccine strain. Therefore, a new vaccine strain selected and changed to A/Sakolnakorn/97 in late 1997 and then to A118/ 87 in the beginning of year 2002 up to the present. As a consequence, new strains were then selected for vaccine production. This study demonstrated that continued monitoring of the evolution FMDV in Thailand is warranted as there may be discrepancy between the field and the vaccine strains. This study would be useful in supporting the selection of appropriate virus vaccine strain for production of high efficacy vaccine and also supporting the control of FMD in Thailand.

Keywords: foot and mouth disease virus, r-value, LP ELISA

Introduction

Foot and mouth disease (FMD), a highly contagious disease of cloven-hoofed animals, is important in Thailand. Seven serotypes of FMD viruses (A, Asia1, C, O, SAT1, SAT2 and SAT3), which do not elicit cross protection, have been identified. Serotypes O, A and Asia1 considered as endemic in Thailand cause 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 can be used to identify serotypes of field samples (Roeder and Le Blance Smith,1987). Other serological tests including virus neutralization (VN) test (Rweyemamu, 1978), liquid phase blocking ELISA (LP ELISA) as well as the virus infection associated agar gel immunodiffusion (VIA-AGID) test (Linchongsubongkoch and Kamolsiripichaiporn.,1990) can be used for disease surveillance and sero-monitoring of both vaccinated and infected animals (Hamblin *et al*.,1986). Recent serological tests with higher specificity and sensitivity utilized recombinant non-structural proteins in indirect ELISA format. These allow the potential for differentiating vaccinated animals from naturally infected ones (Mackey *et al*.,1998). Uses of antibody against the viral capsid proteins can give basic information of the evolution in FMD outbreak strains in the field by investigating the serological relationship (r-value) between virus filed strains and the reference vaccine strains and are useful for the selection of appropriate virus strain for vaccine production. Molecular techniques such as nucleotide sequencing can be used to study genetic relationship between field-isolated viruses and reference vaccine strains with high resolution. These data can be very useful for tracing to the origin of virus causing outbreaks and enhancing the strategic action plan of FMD control at the national and regional level. In this present study, a large group of field isolate viruses (serotypes O, A and Asia1) causing outbreaks in Thailand during 1996-2007 were studied for the evolution of FMDV strains by determining the serological relationship between the reference virus vaccine strains and field isolate viruses. The outcomes will be helpful in selecting appropriate viruses for vaccine production in the future.

Materials and methods

1. Reference viruses and field isolate viruses

Reference viruses type O189/87 (O/Udornthani/87), A118/87(A/Saraburi/1987), A/Sakol/97 (A/Sakolnakorn/1997) and Asia1/85 (Asia1/ Petchaburi/1985) were obtained from current seed vaccine strains. Field specimens from FMD infected animals submitted for laboratory diagnosis were used. They were identified for serotype using double antibody sandwich ELISA test and maintained by inoculating

to primary lamb kidney cell for 2-3 passages and further 4 or 5 passages in BHK-21 cell line. The serotype of the 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 for indirect sandwich ELISA method (Kitching *et al*., 1988) and selected the optimal dilution for virus to use in the liquid phase blocking ELISA (LP ELISA) test (Linchongsubongkoch *et al*., 2000).

2. Bovine antisera

The reference sera used in the LP ELISA, including bovine anti FMD type O189/87, Asia1/85, A/Nakornpathom/87, A/Sakolnakorn/97 and A118/87 were prepared from experimental cattle that have been vaccinated with reference homologous virus. 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. 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 subsequently added to bovine post vaccination serum with homologous virus of each type. 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 vaccine strains which was expressed as r-value (Brookby, 1968).

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

The guideline suggestion for r-value obtained by LP ELISA and criteria of interpretation were described by Samuel *et al*. (1990) and Doughty *et al.*(1995) 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

The situation of FMD outbreak in Thailand during 1997-2007 was studied, recently type O and A have been reported as the most common serotypes, type Asia1 has not been reported since 1999 whereas most of the samples collected from cattle, buffaloes and pigs and less have been collected from sheep and goats as shown in table 1. Consider revision the further strain characterization of filed outbreak viruses was investigated by studying the serological relationship (r-value). R-values type O, A and Asia1 were shown in Tables 2, 3, 4, 5 and 6, respectively.

Table 1. Situation of FMD outbreak in Thailand during 1997-2007, diagnostic assay using standard ELISA typing test to identify FMD serotypes in specimens submitted to the Regional Reference Laboratory.

Year	Total number of samples	Type identification by ELISA typing			
		O	A	Asia1	Not Typed
1997	133	58	45	3	27
1998	144	40	58	2	44
1999	51	17	17	0	17
2000	109	79	8	0	22
2001	162	100	24	0	38
2002	121	37	51*	0	33
2003	123	43	52	0	28
2004	91	19	34	0	38
2005	92	56	21	0	15
2006	85	7	53	0	25
2007	35	15	11	0	9

Note: The first outbreak of new FMD type A occurred in Sakolnakorn province in the end of 1997, and the second outbreak of the same type A occurred in Ratchaburi province in the early 2002.

Table 2. Result of r-value of FMDV type O field isolates in Thailand during 1997-2007, using O189/87 as a homologous system.

Year	Total	% r-value range of type O		
	sample	0-0.19	0.20-0.39	0.40-1.0
1997	44	0(0%)	1 (2.27%)	43 (97.73%)
1998	19	0(0%)	0(0%)	19 (100%)
1999	24	0(0%)	1(4.17%)	23(95.83%
2000	30	0(0%)	4(13.33%)	26(86.67%)
2001	26	0(0%)	0(0%)	26(100%)
2002	6	0(0%)	0(0%)	6(100%)
2003	29	0(0%)	0(0%)	29 (100%)
2004	13	0(0%)	0(0%)	13 (100%)
2005	28	0(0%)	0(0%)	28 (100%)
2006		ND		
2007	5	0(0%)	0(0%)	5 (100%)
	224	0(0%)	6 (2.7%)	218(97.3%)

ND = Not Done, due to field virus could not be adapted into cell culture

Note: O189/87 = O/Udornthani/1987 has been used as seed vaccine strain for type O

Table 3. Result of r-value of FMDV type A field isolates in Thailand during 1997-1998, using A/ Nakornpathom/87 as homologous system.

Year	Total	% r-value range of type A/Nakornpathom/87		
	sample	0-0.19	0.20-0.39	0.40-1.0
1997	9	8 (88.9%)	1 (11.1%)	0 (0%)
1998	4	3 (75%)	1 (25%)	0 (0%)
	13	11 (84.6 %)	2 (15.4 %)	0 (0%)

Table 4. Result of r-value of FMDV type A field isolates in Thailand during 1997-2007, using A/Sakolnakorn/97 as homologous system

Year	Total sample	% r-value range of type A/Sakolnakorn/97		
		0-0.19	0.20-0.39	0.40-1.0
1997	9	0 (0%)	0 (0%)	9 (100%)
1998	4	0 (0%)	0 (0%)	4 (100%)
2001	6	6 (100%)	0 (0%)	0 (0%)
2002	6	6 (100%)	0 (0%)	0 (0%)
2003	35	2 (5.7%)	1 (2.9%)	32 (91.4%)
2004	24	3 (12.7%)	2 (8.33%)	19 (79.17%)
2005	14	0 (0%)	4 (2.8%)	10 (71.42%)
2006	12*	--*	--*	1
2007	10*	--*	--*	--*
	120	17 (17.34 %)	7 (7.14%)	74 (75.51%)

* = Isolate viruses could not have binding reaction with A/Sakolnakorn/97 reagent system

Table 5. Result of r-value of FMDV type A field isolates in Thailand during 1997-2007, using A118/87 as a homologous system.

Year	Total sample	% r-value range of type A/118/87		
		0-0.19	0.20-0.39	0.40-1.0
1997-2000		Not Done		
2001	6	0 (0%)	0 (0%)	6 (100%)
2002	6	0 (0%)	0 (0%)	6 (100%)
2003	35	0 (0%)	1 (2.9%)	34 (97.14%)
2004	19	0 (0%)	1 (5.26%)	18 (94.74%)
2005	14	0 (0%)	4 (28.57%)	10 (71.43%)
2006	16	0 (0%)	1 (6.25%)	15 (93.75%)
2007	13	0 (0%)	0 (0%)	13 (100%)
	109	0 (0%)	7 (6.4%)	102 (93.6%)

A/118/87 = A/Saraburi/1987, classified as A15 BKK60 related group, used as seed vaccine strain since 2001 up to the present

Table 6. Result of r-value of FMDV type Asia1 field isolates in Thailand during 1990-1997, using Asia1/Petchaburi/85 as a homologous system.

Year	Total	% r-value range of type Asia1		
	sample	0-0.19	0.20-0.39	0.40-1.0
1990-1994	47	0(0%)	0(0%)	47 (100%)
1995	18	0(0%)	1(5.56%)	17(94.44%)
1996	13	0(0%)	0(0%)	13(100%)
1997	4	0(0%)	0(0%)	4(100%)
	82	0(0%)	1 (1.2%)	81 (98.2%)

Note: Asia1/Petchaburi/1985 used as seed vaccine strain for type Asia1 up to the present

Discussion

Table 2 shows that 97.3% of field viruses type O collected during 1997-2007 gave an r-value greater than 0.4, indicating that there was a very close serological or antigenic relationship to vaccine strain O189/87 (Linchonsubongkock *et al*., 1992 ; Linchonsubongkoch *et al*., 2000 ; Udon and Linchonsubongkoch, 2006). This finding further indicates that the strain O189/87 was capable of protecting against the majority of type O currently circulating in Thailand. Table 6 demonstrates that 98.2% of field viruses type Asia1 gave an r-value greater than 0.4, further suggesting the relatedness to the reference vaccine strain Asia1/85. This finding indicates that no significant change in the antigenic characteristic of type Asia1 was occurred. Therefore it suggested that there is no need to change vaccine viruses, type O and Asia1, provided that there has been no Asia1 causing outbreak since 1999.

The situation of type A was different. The serological relationship was not investigated during 1993-1996 because no type A outbreaks were reported during that time. Type A was found again at the end of 1997 as shown in Table 3. Interestingly, 84.6% of field viruses type A gave an r-value less than 0.19, while the remaining 15.4% gave an r-value that range 0.20-0.39 when compared with vaccine strain A/Nakornpathom/87 (Linchongsubongkoch *et al*., 2000). This finding indicates that serological relationship varied from most field isolates and correlated with the result from World Reference Laboratory (data not published). Therefore, a new strain isolated from Sakolnakorn province in 1997 was selected and has been using for type A vaccine production. Further investigation of type A viruses collected during 2001-2002 was carried out using A/Sakolnakorn/97 as a homologous reference virus. The finding in Table 4 and 5 showed that 100% of field isolates gave an r-value less than 0.19. However, these field isolates gave an r-value greater than 0.4 when A118/87 was used as the homologous reference

virus. This study indicated that type A isolates from that period have antigenic changed from A/Sakolnakor/97 to A118/87. Taking all of these information into account, a new strain, A118/87, was selected to use for type A vaccine production (Kamolsiripichaiporn and Udon., 2006). This isolate had been collected from cattle in Saraburi province in 1987 and classified to be the same group with A/Bangkok/1960 using monoclonal profiles (Doughty *et al*., 1995). Meanwhile the investigation results of type A during 2003-2005 in Table 4 and 5 showed that 71.42-91.4% and 71.43-97.14% of field viruses using A/Sakolnakorn/97 and A118/87 as homologous reference viruses gave an r-value greater that 0.4 respectively. This indicated that field viruses causing outbreak at that period had close antigenic relationship to both A/Sakolnakorn/97 and A118/87 vaccine strain, hence the vaccine produced by those vaccine strains could give a wide broader protection for most type A field outbreak. However, the field outbreak of type A during 2006-2007 were closely related to current A118/87 vaccine strain. This information is useful for the investigation study of FMDV evolution in Thailand. The antigenic characterization also ensures that the vaccine strains share antigenic similarity with the field strains. In addition, this information is useful for enhancing the strategic action plan for FMD control in Thailand and the Southeast Asia region.

Conclusion

The evolution of FMDV strains was investigated by determining the serological relationship between the reference viruses and field isolate viruses, the r-value of type O and Asia1 indicated that the serological relationship was very close to virus vaccine strain, while type A showed antigenic changes that may be resulting from disease outbreak occurred from time to time.

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วิวัฒนาการทางแอนติเจนของสายพันธุ์เชื้อไวรัสโรคปากและเท้าเปื่อยในประเทศไทย

วิไล ลินจงสุบงกช ร่มพฤกษ์ อุดล และจรรยา สมานิตย์

ศูนย์อ้างอิงโรคปากและเท้าเปื่อย ภูมิภาคเอเชียตะวันออกเฉียงใต้
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บทคัดย่อ

การตรวจจำแนกชนิดไวรัสโรคปากและเท้าเปื่อยจากตัวอย่างสัตว์ป่วยด้วยวิธี ELISA Typing ซึ่งเป็นวิธีมาตรฐานสำหรับการตรวจวินิจฉัยโรคปากและเท้าเปื่อยทางห้องปฏิบัติการในประเทศไทย เชื้อไวรัสโรคปากและเท้าเปื่อยดังกล่าวจะถูกนำมาศึกษาการเปลี่ยนแปลงของสายพันธุ์เชื้อด้วยวิธี Liquid phase blocking ELISA (LP ELISA) โดยทำการหาค่าความสัมพันธ์ทางซีรัมวิทยาหรือ r-value ได้จากการหาสัดส่วนระหว่าง ซีรัมไตเตอร์ที่ได้จากไวรัสจากพื้นที่และซีรัมไตเตอร์ที่ได้จากไวรัสที่ใช้ผลิตวัคซีน จากการศึกษาการเปลี่ยนแปลงของไวรัสโรคปากและเท้าเปื่อย type O, A และ Asia1 ที่ระบาดในพื้นที่ในช่วงระยะเวลา 11 ปีที่ผ่านมา พบว่า type O และ Asia1 ไม่มีการเปลี่ยนแปลงคุณสมบัติทางแอนติเจนจากไวรัสที่ใช้ผลิตวัคซีนในปัจจุบัน จึงไม่มีความจำเป็นในการคัดเลือกไวรัสสายพันธุ์ใหม่สำหรับการผลิตวัคซีนของ type O และ Asia1 (O/189/87 และ Asia1/85 ตามลำดับ) ส่วนกรณี type A พบว่ามีวิวัฒนาการเปลี่ยนแปลงคุณสมบัติทางแอนติเจนไปจากไวรัสที่ผลิตวัคซีน จึงจำเป็นต้องมีการคัดเลือกไวรัสสายพันธุ์ใหม่ที่เหมาะสมสำหรับใช้ในการผลิตวัคซีน ซึ่งได้คัดเลือก A/Sakolnakorn/1997 เพื่อใช้เป็น seed virus สำหรับผลิตวัคซีน type A ในปลายปี 2540 และทำการคัดเลือกไวรัสสายพันธุ์ใหม่อีกครั้งคือ A118/87 เป็น seed virus สำหรับการผลิตวัคซีนในปี 2545 จนถึงปัจจุบัน ข้อมูลดังกล่าวแสดงให้เห็นว่าสายพันธุ์ไวรัสโรคปากและเท้าเปื่อยที่ระบาดในประเทศไทยโดยเฉพาะ type A มีวิวัฒนาการเปลี่ยนแปลงคุณสมบัติทางแอนติเจนไปจากเดิมค่อนข้างมากเมื่อมีการระบาดในแต่ละครั้ง นับเป็นสิ่งสำคัญและจำเป็นอย่างยิ่งต้องมีการศึกษาอย่างต่อเนื่องเพื่อใช้เป็นข้อมูลสนับสนุนการคัดเลือก seed virus ที่เหมาะสมสำหรับผลิตวัคซีนที่มีประสิทธิภาพสูงและสนับสนุนการควบคุมและกำจัดโรคปากและเท้าเปื่อยในประเทศไทย

คำสำคัญ: ไวรัสโรคปากและเท้าเปื่อย r-vale LP ELISA

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