FMD antigenic profiling ELISA

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Abstract

An antigenic profiling of FMD field isolate virus was carried out by double antibody sandwich ELISA using polyclonal antibodies. Totally 50 samples of FMD type A field virus isolates causing outbreak in Thailand and Lao PDR during 2005-2007 and 2011 were studied. The aim of this study was to analyze the antigenic binding reactivity of field virus isolates and compare with several reference virus vaccine strains in order to select an appropriate ELISA reagent system that would be used in further investigation or other research such as vaccine matching test. The result of antigenic profiling test demonstrated that most of FMD type A field isolates from Thailand and Lao PDR during 2005-2007 gave a high antigenic binding reactivity to the reference virus vaccine strain A118/87 rather than A/Sakolnakorn/97, A_{22} Irag 24/64 and A132/87 respectively. Meanwhile, the result of antigenic profiling test of FMD type A field virus isolates from Thailand in 2011 demonstrated that all viruses gave very high antigenic binding reactivity to the reference virus vaccine strain A/Sakolnakorn/97 rather than A118/87 and A132/87 respectively. In conclusion, the recent FMD type A virus outbreak in Thailand in 2011 was categorized as the most A/Sakolnakorn/97 viral related group rather than A118/87 or A132/87. This investigation was useful for preliminary study of viral grouping by antigenic binding reactivity principle in selecting a high specific reagent that would be used in the assay system. Hence, an ELISA reagent of A/Sakolnakorn/97 system was recommended to use in further investigating of vaccine matching test for FMD type A field virus isolate causing outbreak in 2011.

Keywords: foot and mouth disease virus, antigenic profiling, LP ELISA

Background

Foot and mouth disease (FMD) is a highly infectious viral disease of cloven-hooved livestock and is important in Thailand. Susceptible animals which include cattle, water buffalo, sheep, goats, pigs, and wildlife. The epidemiology of FMD have resulted in the slaughter of millions of animals, despite this being a frequently nonfatal disease for adult animals, though young animals can have a high mortality. FMD viruses isolated from different outbreaks showed much antigenic difference, which generally results in vaccine lacking effective protection against those FMD epidemic strains and even causing FMD outbreak. Therefore, the epidemiological program to assess regularly the antigenic characteristic of field isolates.The monitoring of the antigenic relationship of field isolates in relation to the reference vaccine strain can be show the efficacy of the vaccine virus in use and also has access to select the most suitable vaccine strains in case of vaccine selection. (Ma et. al.(2011). Linchongsubongkoch et.al.(2008), reported that FMD type A field isolated viruses indicated an antigenically changed from virus vaccine strain from time to time and associated with the re-circulation of serotype A in the field causing problem for the control of field virus through the

appropriate vaccine. Knowledge on the antigenicity of circulating viruses was important to select most suitable vaccine strains and ensure prompt identification of new variants. Hence the selection of suitable and specific diagnostic reagents corresponding to the current virus outbreak strain become an very important in assay system such as antigen titration, vaccine matching or other research purpose. An antigenic profiling ELISA was carried out in antigen titration process by using polyclonal antisera which was suitable approach for selecting an appropriate ELISA reagent system for further investigation of vaccine matching or other research studies. This study was to analyze the antigenic binding reactivity of field isolate viruses serotype A causing outbreaks in Thailand and Lao PDR during 2005-2007 and Thailand in 2011 comparing with several reference virus vaccine strains. The result would be calculated in percent binding reaction between test sample and reference virus.

Materials and methods

Reference viruses and field viruses

Reference viruses type A118/87, A132/87, A_{22} Iraq 24/64 and A/Sakolnakorn/97 were obtained from seed vaccine strains, except for A_{22} Iraq 24/64 was provided from World Reference laboratory for FMD (WRL), Pirbright Laboratory, UK. Field samples from FMD infected animals submitted for laboratory diagnosis which were from Lao PDR, Thailand during 2005- 2007 and Thailand in 2011 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 viral 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 used in this study.

Antigen titration by ELISA technique

Antigen titration was carried out by double antibody sandwich ELISA method as this following. 96-well ELISA plate was coated by rabbit trapping antiserum raised against FMD type A118/87, A132/87, A_{22} Iraq 24/64 and A/Sakolnakorn/97 in separated plate for each serotype at dilution 1:5000 in Carbonate/bicarbonate buffer (Sigma). Incubate at +4°C overnight. Prepare two fold dilution series of virus isolation fluid and reference virus (1:2, 1:4, 1:8, 1:16, ..., 1:128) in PBST buffer , 50 ul/well was added from row A to G, row H for blank, sample no. 1-10 was added in column 1-10, and column 11-12 for control reference virus. Plate were incubated at 37 °C incubator on rotary shaker for 1 hour, wash plate 4 times. Guinea pig detecting antisera against FMD type A118/87, A132/87, A_{22} Iraq 24/64 and A/Sakolnakorn/97 in blocking buffer were prepared and added to each corresponding plate, incubated at 37°C 1 hour, then wash as before. Horseradish peroxidase conjugate was added across the plate, incubated at 37°C 1 hour, then wash as before. TMB substrate was added, leave at room temperature for 20 minutes, then stop reaction with 1 M H_2SO_4. The color reaction was measure by reading optical density (OD) of ELISA reader at wave length 450 nm.

Antigenic binding reactivity

Antigenic binding reactivity was expressed as percentage of binding reactivity (% binding) between the OD of test sample and OD reference virus at the antigen dilution where as an OD in range 1.0-1.5, in this assay system, the appropriate dilution used in calculating percentage of binding reactivity was at 1:16 after subtract of background (Bg) in each plate.

$$\% \text{ binding reactivity} = \frac{\text{OD sample-OD Bg}}{\text{OD reference virus-OD Bg}} \times 100$$

Results

All field isolate virus and reference vaccine virus used in this studied was listed in table 1. The result of antigenic profiling test of FMD type A field isolates from Thailand and Lao PDR during 2005-2007 compared with reference vaccine strain of A132/87, A_{22}Iraq 24/64, A/Sakolnakorn/97 and A118/87 were shown in figure1 , 2, 3 and 4 respectively.

Table1. List of FMD field isolate viruses and reference virus vaccine strain used in this studied in Thailand and Lao PDR during 2005-2007 and 2011

Sample number	Sample Name	Sample number	Sample name
1	THA 2/07	26	A 65/11
2	THA 87/06	27	A 67/11
3	THA 31-1/05	28	A 68-1/11
4	THA 76/06	29	A 69-1/11
5	THA 84/06	30	A 70-1/11
6	THA 16/06	31	A 70-2/11
7	THA 46-1/05	32	A 71-1/11
8	THA 80/05	33	A 71-2/11
9	THA 50/06	34	A 73-2/11
10	THA 78/06	35	A 74/11
11	Reference reference vaccine virus of each strain	36	A 75/11
12	A 8/11	37	A 76-1/11
13	A 10-2/11	38	A 77-2/11
14	A 16-3/11	39	A 78/11
15	A 17-2/11	40	A 79-1/11
16	A 19/11	41	A 80-3/11
17	A 25-2/11	42	A 65/11
18	A 29-1/11	43	A 67/11
19	A 53-1/11	44	A 68-1/11
20	A 53-2/11	45	A 69-1/11
21	A 57-2/11	46	A 70-1/11
22	A 58/11	47	A 70-2/11
23	A 59-2/11	48	A 71-1/11
24	A 63/11	49	A 71-2/11
25	A 64/11	50	A 73-2/11

Figure1. Result of % binding reactivity of FMD type A field isolate viruses from Thailand during 2005-2007 to the reference virus vaccine strain A132/87, A_{22}Iraq 24/64, A/Sakolnakorn/97 and A118/87. The result showed a high antigenic binding reactivity to the reference virus vaccine strain A118/87 rather than A/Sakolnakorn/97, A_{22} Irag 24/64 and A132/87 respectively.

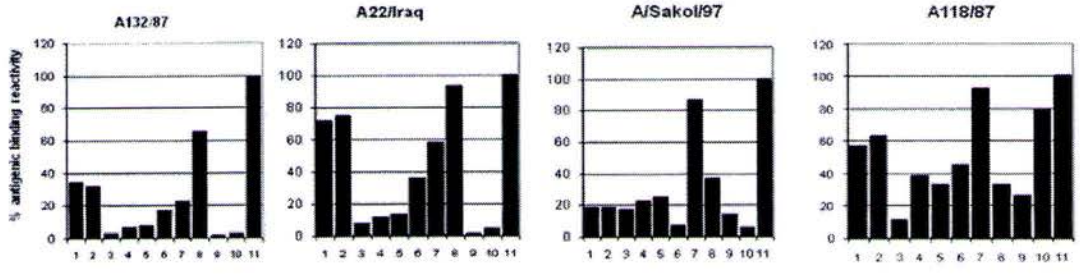


Figure 2. Show the percentage of binding reactivity field isolate virus in Thailand in 2011 react with Reference vaccine strain A118/87 system, all samples gave <40 % binding reactivity

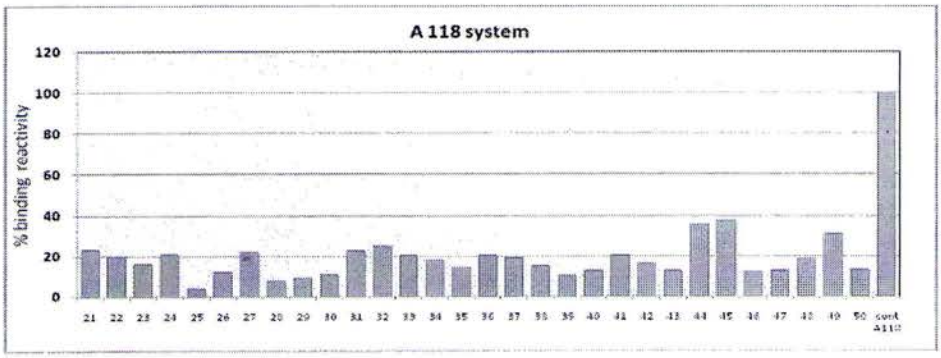


Figure 3. Show the percentage of binding reactivity field isolate virus in Thailand in 2011 react with Reference vaccine strain A/Sakolnakorn/97 system, majority gave 50-80% binding reactivity.

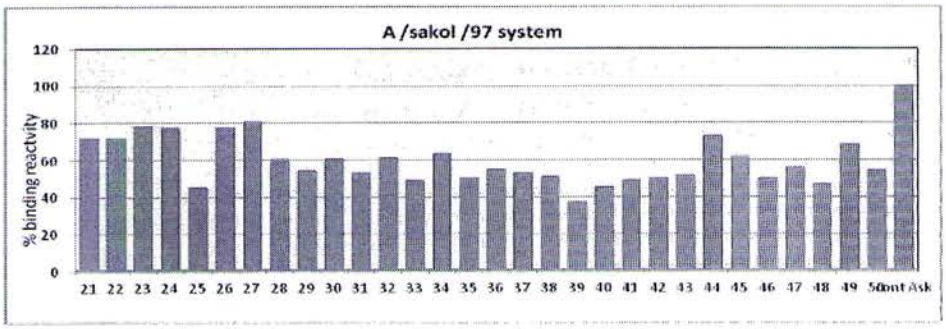
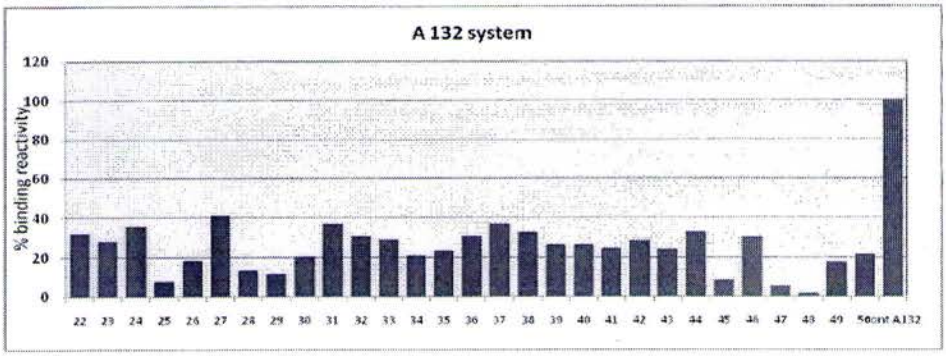


Figure 4. Show the percentage of binding reactivity field isolate virus in Thailand in 2011 react with Reference vaccine strain A132/87 system, all samples gave <40 % binding reactivity.



Conclusion

The antigenic profiling ELISA of FMD type A field isolate viruses from Thailand and Lao PDR during 2005-2007, majority was given a high antigenic binding reactivity to the reference virus vaccine strain A118/87 rather than A/Sakolnakorn /97, A_{22} Irag 24/64 and A132/87 respectively. In contrast, most field isolate viruses type A causing outbreak in Thailand in 2011 indicted the very high antigenic binding reactivity to the reference virus vaccine strain A/Sakolnakorn/97 (>50-80% binding reactivity)) rather than A118/87 (< 40% binding reactivity) and A132/87 (<40% binding reactivity) respectively, it mean that filed isolate virus showed remarkable close antigenic reactivity to the homologous virus (Samuel et. al,1991). However, Aggarwal et al.(2011) reported that antigenicity of FMDV could be changed because of frequent mutations in it's genome, thus

evade the protective immunity provided by vaccine. Therefore, other research study was needed to support that occurrence. An antigenic profiling could provide a rapid indication of vaccine matching investigation and this results motivated by the necessity of large-scale immunological thoroughly. Figure 1 showed that propensity of antigenic binding reactivity of FMD type A field isolate viruses from infected samples of Thailand and Lao PDR during 2005-2007 were remarkable as high antigenic binding reactivity to the reference virus vaccine strain A118/87 rather than A/ Sakolnakorn/97, A_{22} Irag 24/64 and A132/87 respectively. Furthermore, the field outbreak of type A during 2006-2007 were closely related to type A 118/87 vaccine strain (Linchongsubongkoch et al., 2008). However, in 2011 the antigenic profiling result was demonstrated high % binding reactivity to A /Sakolnakorn/97 system than other as shown in figure 2, 3 and 4. Interestingly, most of type A field outbreak in Thailand in 2011 were demonstrated the high antigenic binding reactivity with A/ Sakolnakorn/97 system (> 50-80% binding reactivity as showed in figure 3) and less binding reactivity to A118/87 and A132/87 (<40% binding reactivity as showed in figure 2 and 4) . This result could be provided the preliminary basic information of viral grouping in selecting an appropriate ELISA reagent to use further vaccine matching.

In conclusion, antigenic profiling study of FMD type A from Thailand and Lao PDR during 2005-2007 was indicated that most of field viruses gave a high binding reactivity to the vaccine strain A118/87 while the FMD type A in 2011 was indicated that most of field viruses gave high binding reactivity to A/Sakolnakorn/97. This investigation was useful for preliminary study of antigenic grouping in basic principle for selecting a high specific reagent that would be used in the diagnostic assay system. Hence, an ELISA reagent of A/Sakolnakorn/97 system was recommended to use in further investigating of vaccine matching test for FMD type A filed isolate virus causing outbreak in 2011.

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