Development of an ELISA for Antibody to a FMDV Non-Structural Protein Using a Chemically Synthetic Peptide as Antigen

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Introduction

Current serological tests to detect antibody to FMD virus include virus neutralization (SNT), liquid-phase blocking (LPB) ELISA, solid-phase competition ELISA and nonstructural protein (NS) ELISA or equivalents (1, 2). The advantage of NS as ELISA antigen is not only the group specific characteristics of the antigen, but also the specificity for so-called 'virus infection' antibodies. The latter characteristic was expected to provide a possible tool, differentiating infected from vaccinated animals. These advantages have been demonstrated using biologically engineered recombinant NS antigens (3, 4). Our objective was to develop an NS peptide antigen for NS ELISA, which could replace the recombinant NS antigens.

Materials and Methods

NS Peptide Antigens: FMDV NS peptides were synthesized by Beckman 990B and Advance ChemTeck ACT90 using F-moc chemistry. They were then conjugated with horse gamma globulin (Chon fraction II & III, Sigma) by a modified glutaraldehyde method (5).
Sera: Sera collected from Japanese healthy dairy cattle in 1996 were used as FMD sero-negative sera after confirmation of their sero-negative status by SNT and LPB ELISA. Individual convalescent sera derived from all types of FMDV infected cattle were obtained from IAH Pirbright and used as reference sera. Serially collected sera, which derived from four experimentally infected cattle (#198, #200, #203, #214 infected with O/ Udornthani/ 87), were also used as positive sera.
NS Peptide ELISA: Indirect ELISA was performed. Peptides–horse gamma globulin conjugates (10ug/ml) were used as antigen and coated directly onto plates with carbonate buffer pH9.6.
NS ELISA, SNT and LPB-ELISA: These assays were performed following standard protocols.
Gene analysis: GENETYX software was used with FMDV-O1K sequence (X00871) (6).

Results and Discussion

Screening of Peptides: Forty peptides (9AAs to 28AAs long), covering all of the hydrophilic peaks on the NS region of FMDV sequence, were synthesized as according to Materials and Methods. They were then subjected to ELISA to test their activity as antigens using #214 convalescent serum (4wk after challenge). Finally, the #7 peptide (Table.1), which showed the highest activity, was selected as the candidate NS peptide antigen (Data not shown).

Table 1: Sequence of #7 peptide

Location (AAs)	Sequence	NS region
1094-1107 (13)	RSTPEDLERAEKQ	2B

Establishing the Cut-off: One hundred negative sera were subjected to NS peptide ELISA at various dilutions. At a dilution of 1:400, the mean OD values in ELISA was 0.05 (n=100, Max; 0.15, Min; -0.09, SD=0.13). The cut-off was consequently set provisionally at 0.20 OD at a dilution of 1:400. Two fold serum dilutions were made from 1:400. Sera with an OD greater than 0.2 at 1:400 were considered positive and the titer was defined as the last dilution with an OD >0.2
Positive Reference Sera: All the reference sera subjected to ELISA were positive (1:800 to 1:51200).
Serially collected Sera from Infected Cattle: The NS peptide ELISA was compared with NS ELISA, SNT and LPB-ELISA using serially collected sera from experimentally infected cattle. The NS peptide ELISA showed a good correlation with other serological tests (Table2. showed the results of animal #200). These results suggest that peptide #7 may represent a viable alternative to recombinant NS antigens and would have advantages in terms of ease and consistency of production, stability and cost.

Acknowledgments

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References

1. Manual of Standards for Diagnostic Tests and Vaccine, 2000. OIE
2. Mackay et al., 2001. J Virol Methods 97 : 33-48
3. Diego et al., 1997. Arch Virol 142 : 2021-2033
4. Mackay et al., 1998. Vaccine 16 : 446-459
5. Zegers et al., 1990. J Immunol Methods 130 : 195-200
6. Forss et al., 1984. Nucleic Acids Res 12 : 6587-6601

Table 2: Comparison of serological tests using serially collected sera from experimentally infected cattle

Sera (#200)	NS Peptide ELISA	NS ELISA	VNT (O/Udorn/87)	LPB-ELISA (O/Udorn/87)
0 wk*	- (<400)	- (0.07) **	<1	<22
1 wk	+ (3200)	+ (0.41)	80	1445
2 wks	+ (51200)	+ (1.52)	640	2884
3 wks	+ (51200)	+ (1.44)	320	2884
4 wks	+ (12800)	+ (1.25)	320	2884
5 wks	+ (12800)	+ (1.19)	320	1445
6 wks	+ (3200)	+ (0.88)	160	1445
7 wks	+ (3200)	+ (1.02)	160	1445
8 wks	+ (3200)	+ (0.78)	160	1445
9 wks	+ (3200)	+ (0.55)	160	2884
10 wks	+ (800)	+ (0.40)	160	2884

* Week(s) after challenge, ** T/P value