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Development of a peptide based latex agglutination assay for serotype identification of foot and mouth disease virus

Dilpreet Kaur, Gurpreet Kaur*, Mudit Chandra, Hari M Saxena & Padam N Dwivedi

Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India

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Out of 200 serum samples collected from cattle (142) and buffaloes (58) of various ages and sexand subjected to latex agglutination test (LAT) using serotype specific peptides (O, A, Asia 1) and also with peptide for non-structural protein 2B (NSP-2B), 114 (70%) samples were positive against FMDV type 'O', 102 (51%) against serotype 'A' and 104 (52%) against serotype 'Asia 1'. With NSP-2B peptide a total of 71 (35.5%) samples were positive. The results suggest that LAT could be used for the diagnosis of foot and mouth disease virus as it is easy, cheap and effective test.

Keywords: FMD virus, Latex agglutination test, Non-structural protein 2B, Serotype specific peptides

Foot and mouth disease (FMD) is a highly contagious disease of cloven footed animals. It is one of the most important viral infections affecting bovine that affects economy of livestock worldwide[1,2].

The genome of FMD virus encodes a single protein that is cleaved to form mature products which include four structural proteins viz. VP1, VP2, VP3, and VP4 and eight non-structural proteins viz.2B, 2C, 3A, 3D, L^{pro}, 2A, and $3C^{pro3-5}$. Among the structural proteinsVP1 is the most frequently studied protein owing to its significant roles in serotype specificity, virus attachment and protective immunity. The high sequence variability found in this protein accounts for the low cross reactivity observed among different serotypes[6]. It is also known that both the cell attachment site and the immunodominant region of FMD virus are located on a solvent exposed region at the surface of the virion, namely the trypsin-sensitive areas of VP1[7,8]. Also important antigenic sites of FMD virus are located on the sequence between amino acids 140 and 160 and that of the C terminus of VP1[9-11] and several overlapping B-cell epitopes located within this region are able to induce both neutralizing and non-neutralising antibody responses[6].

Several diagnostic tests such as complement fixation test (CFT), enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are used in diagnosis of FMDV but none of them is applicable in the field conditions due to their requirement of specialised laboratories, skilled personnel and high cost. Serotype specific peptides have been used for the diagnosis of FMDV[12] but these had limited field application as the activity of the tested peptides were studied using solid-phase indirect radioimmunoassay on polyethylene film with purified immunoglobulins against intact FMD virus.

The present study has been undertaken to use latex agglutination test (LAT) as an alternative diagnostic method in the diagnosis of FMDV and also to use it as differentiation of infected from vaccinated animals (DIVA) when peptides against non-structural protein were incorporated in the test.

Materials and Methods

Collection of sera—A total of 200 blood samples were collected from cattle (142) and buffaloes (58) in and around Ludhiana District, Punjab from December 2010 to April 2011. Out of 142 cattle, 5 (3.52%) were under 6 months of age, 8 (5.63%) were from 6-12 months of age, 11 (7.74%) were 1-2 years old and 118 (83.09%) were more than 2 years of age. Out of 58 buffaloes, 4 (6.89%) were 1-2 years old and 54 (93.10%) were of more than 2 years of age. The vaccination status of the individual animal was also recorded as informed by its owner. Serum was separated from the clotted blood and was stored at -20 °C till further use.

*Correspondent author
Telephone: 91-161-2414033
E-mail: gurpreet7502@rediffmail.com

Selection of serotype specific sequences—For the identification and selection of serotype specific peptide sequences, already published sequences differentiating different FMDV serotypes were selected[13,14]. Basic local alignment search tool (BLAST) software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the selected viral peptide sequences with the FMDV sequences available in the Genbank (www.ncbi.nlm.nih.gov/genbank). To further increase serotype-specificity the above sequences were trimmed to prevent any cross reactions or non specific agglutination reactions between serotypes during the test.

The amino acid residues 140-160 from VP1 of A_{24}Cruzeiro and 'O_1'Kaufbeuren strains of FMDV were selected for peptide synthesis. The amino acids residues 140-160 and 200-213 from the VP1 sequence of FMD type 'Asia 1'(Asia 1 Ind 63/72) were selected for peptide synthesis and both the proteins short chains were linked using proline-proline-serine (P-P-S)[13]. Non-structural protein (NSP) 2B of FMD was selected from the serotype 'O1K' for the peptide synthesis[14].

The amino acid sequences selected were as follows:

a. A (A_{24}Cruzeiro): GSGRRGDMGSLAARVVKQ
b. O (O_1 Kaufbeuren): VPNLRGDLQVLAQKVART
c. Asia 1 (Asia 1 Ind63/72): RRKQEIIAPEKQVLPPSQPTRRGD LAVLAQRVSNR
d. NSP 2B (O1K): RSTPEDLERAEKQ

Synthesis and conjugation of peptides—The peptides 'O', 'A', 'Asia 1' and NSP 2B, were coated with deep blue, white, dark red and fluorescent orange coloured latex beads (Sigma=Aldrich, Germany). The ***invitro*** synthesis of all the individual peptides and its tagging with variously coloured latex beads were done from Bioconcept Labs Pvt Ltd, Gurgaon.

Latex agglutination test—Equal quantity (5 µL) of serum sample and peptide coated latex beads were put on a clean grease free glass slide individually. It was mixed thoroughly using a sterile wooden tooth pick and the formation of clump within one minute was indicative of positive agglutination reaction. Foetal calf serum (Sigma-Aldrich, Germany) was used instead of serum as negative control.

Multiplex latex agglutination test—Serum sample (5µL) and 5 µL each of FMDV type O, A, Asia1 and NSP 2B peptides conjugated latex beads were put together on a clean grease free glass slide. The serum sample and peptides were mixed thoroughly using a sterile wooden tooth pick to observe the clump formation within one minute. Fetal calf serum (Sigma-Aldrich, Germany) was used instead of serum as negative control.

Results and Discussion

Foot and mouth disease is one among the most contagious diseases of livestock and is endemic in India till date. It is prevalent almost in all the parts of the country and occurs round the year. In the present study a total of 200 serum samples collected from cattle and buffaloes in and around Ludhiana district were subjected to LAT using serotype specific peptides against O, A and Asia 1 conjugated with different coloured beads. Since only four serotypes O, A, C and Asia 1 of FMD have been reported from India till date and out of these serotype C has not been reported from India since 1995[15], serotype C was not considered in the present study and was excluded. The subsequence 140-160 amino acid residues of VP1 of A_{24} Cruzeiro and O_1 Kaufbeuren strains of FMDV were selected for peptide synthesis since this particular region is reported to be unique for these serotypes[13]. Though in the present study peptides were synthesized from A_{24} Cruzeiro which is not of Indian origin, upon blasting the selected peptide sequence used in the study it was observed that the selected peptide was able to identify many of the Indian FMDV serotype A.

Similarly the O_1 serotype selected for peptide synthesis in the study was not of Indian origin but it was observed that upon trimming the selected peptide was able to identify more no. of Indian O serotypes with higher specificity when compared by BLAST. However, for Asia 1 alongwith 140-160 amino acid residues[16] of VP1, minor amino acid residues from 200-213 also on VP1 were identified and linked together. Since these minor amino acid residues on VP1 act as a minor neutralizing site for Asia1[10], linking of these two residues together was done to increase the specificity to identify Asia 1.

Latex agglutination test using beads conjugated with serotype specific peptides—Out of 200 sero-samples from cattle and buffaloes when tested with LAT indicated 114 (70%) samples were positive against FMDV type O, 102 (51%) against type A and 104 (52%) against serotype Asia 1 (Fig. 1). These results indicate that the tagging with the peptides was able to identify serotypes successfully, since the current vaccine against FMD manufactured in the

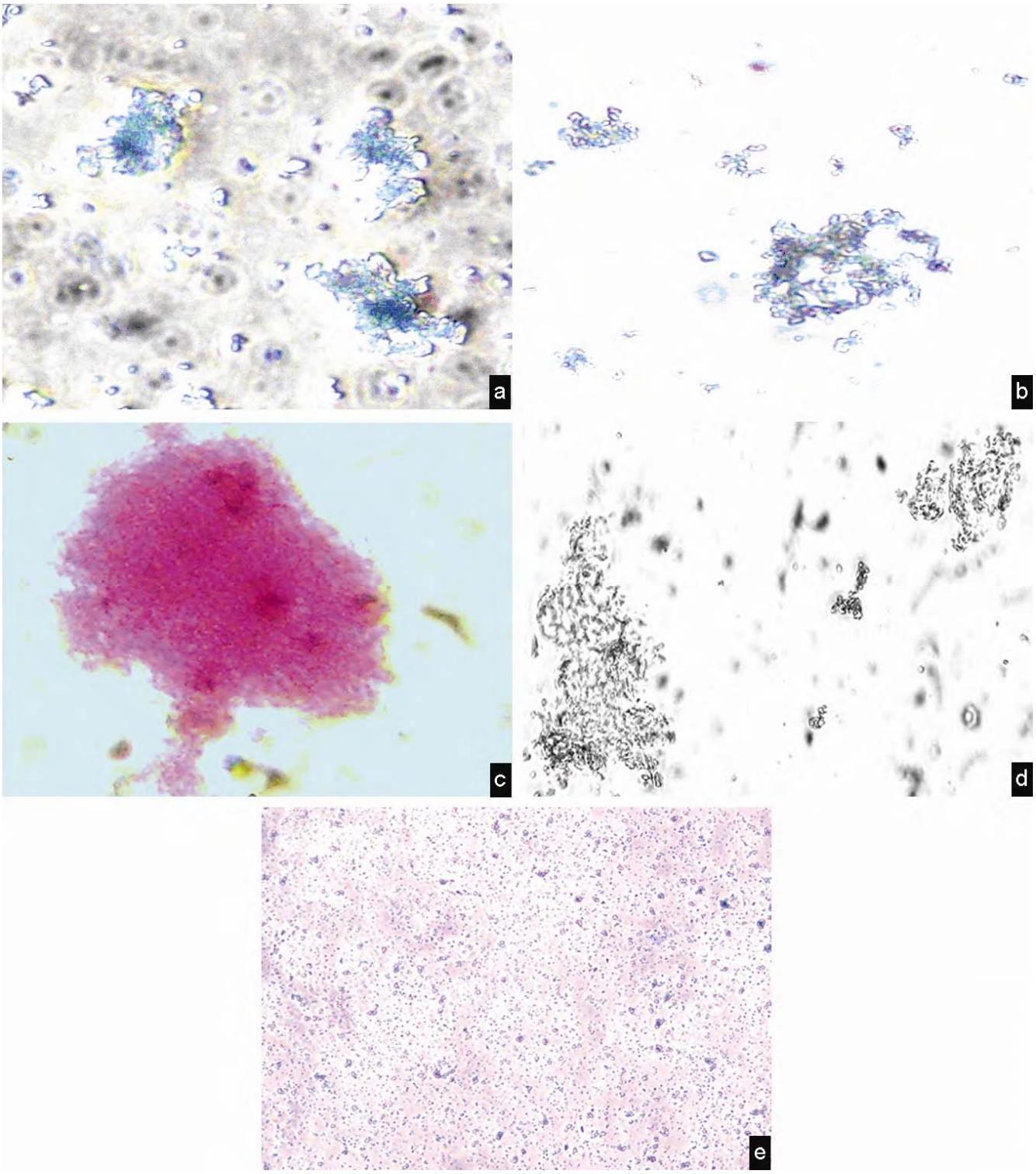

Fig. 1—Agglutination of serum by specific FMDV peptides. a: O (blue beads); b: A (white beads), c: Asia 1(dark red), d: Non-structural peptide (NSP 2B) (fluorescent orange),e: Absence (control FCS)

country is a trivalent vaccine consisting of serotypes O, A and Asia 1[17]. No agglutination was observed with fetal calf serum (Fig. 1). These findings also indicate that the prevalence of antibodies against type O was maximum followed by Asia 1 and A in the test population. The presence of antibodies against O serotype in more number of animals than the other two serotype indicates that the vaccine used in these animals had good antigenic mass against serotype O as compared to the other two serotypes which had almost same percent prevalence in the population when tested with LAT. Moreover, it has also been reported earlier too that about 85% of the outbreaks of FMD in India are caused by serotype O, 8-10% by A and rest due to Asia 1[18] indicating that protection against serotype O is very important from control point of view against FMD (Table 1).

Also, maximum numbers of animals exhibiting antibodies against serotype O are in concurrence with the earlier study in which with LPB ELISA 70.90% serum samples showed protective antibodies against type O, 61.06% against A type and 37.70% against type Asia-1[19]. These results suggest that specific peptide based latex agglutination test is successful in identification of serotypes of FMDV on the basis of agglutination test.

Latex agglutination test using beads conjugated withnon-structural protein (NSP-2B) peptide—When all the serum samples were subjected to LAT using NSP-2B peptide it was found that 71 (35.5%) animals were positive against NSP (Fig. 1). Since antibodies against NSP can only be produced in animals infected with FMD and cannot be produced in animals that are only vaccinated thus it helps in differentiation of infected from vaccinated animals[20]. Also, vaccination against FMD is inadequate in almost all the states due to which occasional disease out-breaks occur in the endemic zones[21]. Therefore, DIVA test is important and assumes a greater significance in the field (Table 1).

Multiplex latex agglutination test—The above test was performed only with limited no. of samples to observe whether the multiplexing using coloured bead is successful. When multiplexing using all the peptides with different latex beads was combined in a single reaction, the agglutination with beads of different types were clearly visible (Fig. 2). The

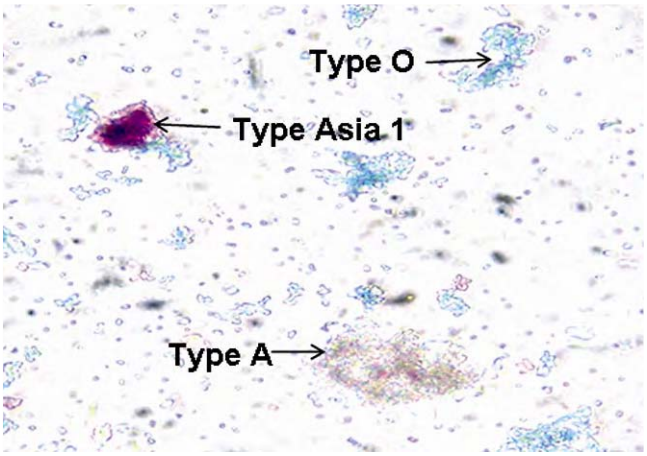

Fig. 2—Simultaneous agglutination of beads conjugated to three different serotypes of FMDV with serum from vaccinated animal

Table 1—Cattle (C) and buffaloes (B) positive for serotype O, A, Asia 1 and NSP

Age groups		Total No. of animals	O			A			Asia 1			NSP		
			Total positive	V	NV	Total positive	V	NV	Total positive	V	NV	Total positive	V	NV
<6 m	C	5	4	4	0	5	5	0	4	4	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0
6-12m	C	8	5	5	0	2	2	0	1	1	0	3	3	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0
1-2 y	C	11	6	5	1	5	3	2	3	3	0	2	2	0
	B	4	2	1	1	2	2	0	4	4	0	3	3	0
>2 y	C	118	65	58	7	55	34	21	58	50	8	40	36	4
	B	54	32	31	1	38	32	6	34	29	5	23	19	4
Total	C	142	80	72	8	67	44	23	66	58	8	45	41	4
	B	58	34	32	2	40	34	6	38	33	5	26	22	4
Percent	C		56.34			47.2			46.5			31.7		
	B		58.6			68.97			65.5			44.83		

V: vaccinated; NV: non vaccinated

results indicate that tagging the peptides with different coloured beads and simultaneously using them in a single reaction could be used for initial screening.

Thus from the present study it could be concluded that out of 200 serum samples subjected to LAT, 114 samples were positive and 86 were negative for antibodies to FMDV type O, against serotype A, 102 samples were positive and 98 were negative and against serotype Asia 1, 104 were positive and 96 were negative. When NSP peptide was used in LAT, 71 samples were positive and 129 were negative indicating that with tagged NSP peptide vaccinated animal could be detected and differentiated from infected animals. Finally LAT is cheap, effective and easy test which can be used for FMDV diagnosis at field, however, further validation is required.

References

1 Kitching R P, Foot-and-mouth disease: Current world situation, ***Vaccine,*** 17 (1992) 1772.

2 Bachrach H L, Foot-and-mouth disease virus, ***Ann Rev Microbiol***, 22 (1968) 201.

3 Carrillo C, Tulman E R, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish G F & Rock D L, Comparative genomics of foot-and-mouth disease virus, ***J Virol,*** 79 (2005) 6487.

4 Belsham G J, Translation and replication of FMDV RNA, ***Curr Top Microbiol Immunol,*** 288 (2005) 43.

5 Mason P W, Grubman M J & Baxt B, Molecular basis of pathogenesis of FMDV, ***Virus Res***, 91 (2003) 9-32.

6 Grubman M J, Zellner M & Wagner J, Antigenic comparison of the polypeptides of foot and mouth disease virus serotypes and other picornaviruses, ***Virology,*** 158 (1987) 133.

7 Cavanagh D, Sangar D V, Rowlands D J & Brown F, Immunogenic and cell attachment sites of foot-and-mouth disease virus: Further evidence for their location in a single capsid polypeptide, ***J Gen Virol,*** 35 (1977) 149.

8 Laporte J & Lenoir G, Structural proteins of foot-and-mouth disease virus, ***J Gen Virol,*** 20 (1973) 161.

9 Pfaff E, Thiel H J, Beck E, Strohmaier K & Schaller H, Analysis of neutralizing epitopes of foot-and-mouth disease virus, ***J Virol***, 62 (1988) 2033.

10 Strohmaier K, Franze R & Adam K H, Location and characterization of the antigenic portion of the FMDV immunizing protein, ***J Gen Virol,*** 59 (1982) 295.

11 Bittle J L, Houghten R A, Alexander H, Shinnick T M, Sutcliffe J G & Lerner R A, Protection against foot-and-mouth disease virus by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence, ***Nature,*** 298 (1982) 30.

12 Petrov V N, Rybakov S S, Petrova O N, Lugovskol A A & Pavlov A V, The use of synthetic peptides for detection of antibodies against foot-and-mouth disease virus in the blood from convalescent animals, ***Mol Gen Microbiol Virusol,*** 3 (1996) 27.

13 Doel T R, Gale C, Do Amaral C M C F, Mulcahy G & Dimarchi R, Heterotypic protection induced by synthetic peptides corresponding to three serotypes of foot and mouth disease virus, ***J Virol***, 64 (1990) 2260.

14 Inoue T, Parida S, Paton D J, Linchongsubongkoch W, Mackay D, Anupomma D, Gubbins S & Saikil T, Development and evaluation of an indirect enzyme linked immunosorbent assay for detection of foot and mouth disease virus non-structural protein antibody using chemically synthesized 2B peptide as antigen, ***J Vet Diagn Invest,*** 18 (2006) 545.

15 Shah K A, Jan F A, Andrabi A & Kaloo F A, Incidence, serotyping and epidemiology of foot and mouth disease in Kashmir, ***Online Vet J***, 6 (2011) 1.

16 Reddy G R, Tratschin J D, Natarajan C & Suryanarayana V V S, Capsid protein encoding (P1) sequence of foot and mouth disease virus Type Asia 1 Ind 63/72, ***Virus Genes,*** 18 (1999) 161.

17 Hemadri D, Sanyal A, Tamil Selvan R P, Saravanan S, Mohapatra J K, Lalkrishna, Bujarbaruah K M & Pattnaik B***, Constraints and Opportunities for the control of Foot and Mouth Disease in India,*** paper presented to The Global control of FMD-Tools, ideas and ideals–Erice, Italy, 14-17 October 2008.

18 Biswal J K, Sanyal A, Rodriguez L, Subramaniam S, Arzt J, Sharma G K, Hammond J M, Parida S, Mohapatra J, Mathapati B S, Dash B B, Ranjan R, Rout M, Venketaramanan R, Misri J, Krishna L, Prasad G, Pathak K M L & Pattnaik B, Foot-and-mouth disease: Global status and Indian perspective, ***Indian J Anim Sci***, 82 (2012) 109.

19 Annual Report, Project Directorate on Foot and Mouth Disease, ICAR, New Delhi, India, 2008-2009.

20 Sorensen K J, Madsen K G, Madsen E S, Salt J S, Nqindi J & Mackay D K, Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus, ***Arch Virol,*** 143 (1998) 1461.

21 Longjam N, Deb R, Sarmah A K, TayoT, AwachatVB & Saxena V K, A brief Review on diagnosis of foot-and-mouth disease of livestock: Conventional to molecular tools, ***Vet Med Int***, (2011) doi:10.4061/2011/905768.