openaccess Journal
Veterinaria

2015 | Volume 3 | Issue 1 | pages 21-25

Research paper

Rapid Detection of Foot and Mouth Disease Virus (FMDV) through Co-agglutination Test

Baitullah Khan[a*], Rafiullah Khan[b], Khawar Ali Shahzad[c, d]

[a]Veterinary Research and Disease Investigation Centre, Hangu Road Kohat, 26000, Pakistan
[b]Department of Poultry Sciences, Agriculture University Peshawar, 25000, Pakistan
[c]Department of Microbiology and Immunology, Southeast University, Nanjing, 210009, China
[d]Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, 54000, Pakistan

Abstract
In present study, a Coagglutination test (COAT) was optimized for rapid detection of Foot and Mouth Disease Virus (FMDV). Immune sera against FMDV O, A and Asia1 strains were raised separately and in combination. Protein A, containing *Staphylococcus aureus* pellet was sensitized with rabbit anti-FMD sera. COAT reagents were tested against laboratory FMD virus. The 1/100 dilution *S. aureus* with 250µl serum was found specific for FMD virus. COAT reagents depicted no agglutination with phosphate buffer saline, cell culture media, animal saliva and PPR virus. Forty vesicular fluid samples were tested with combined conjugate (prepared by using trivalent sera), in which 38 were found positive. Of 38 vesicular fluid (VF) samples tested, 33 of them were O, 3 were Asia1 and 2 were A strains of FMDV. COAT was found 100% sensitive and highly specific for detection of FMD virus in vesicular fluid. As this test was easy to perform it could be used for rapid diagnosis of FMD virus.

Keywords: Coagglutination test, FMDV, Asia 1, Agglutination, Trivalent sera

Received; October 10, 2014 **Revised**; February 16, 2014 **Accepted;** March 4, 2015
***Corresponding author:** Baitullah Khan **Email**: bait.living@gmail.com

To cite this manuscript: Khan B, Khan R, Shahzad KA. Rapid detection of Foot and Mouth Disease Virus (FMDV) through co-agglutination test. Veterinaria 2015; 3(2): 21-25.

Introduction

Foot and mouth disease (FMD) is caused by a virus of the genus Aphthovirus, family Picornaviridae [1]. The virus genome consists of a single stranded positive-sense RNA of approximately 8 kb [2]. There are seven serotypes of FMD virus O, A, C, Asia 1, SAT1, SAT 2, and SAT 3 [3]. The disease can affect animals including cattle, pig, sheep, goats, wild ruminants and water buffaloes [4].

Animal diseases are important factor in lowering productivity of animals. FMDV is endemic in Pakistan [5]. Foot and mouth disease is responsible for significant economic losses [6]. Infectious diseases of farm animals have an inverse impact on food supply, public health and national economy. Approximate losses due to FMDV in 2003 were Rs. 6 billion [7].

Rapid diagnosis is necessary to control foot and mouth disease. Several diagnostic tests such as complement fixation test (CFT), virus neutralization test, enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) already available for diagnosis of FMDV [8, 9]. These tests require skilled manpower, more time, sophisticated labs and high cost. The advantage of coagglutination test is that it is simple, sensitive and rapid [10]. This test is economical both for lab and field for diagnosis of FMDV as compared to the other tests [11]. The aim of the study was to optimize staphylococcal coagglutination test for rapid detection of foot and mouth disease virus and validation of this test in field.

Materials and Methods

The study was conducted in Microbiology Department and WTO Quality operation laboratory (QOL), University of Veterinary and Animal sciences Lahore, Pakistan. Moreover, FMDV vaccine viral strains and *Staphylococcus aureus* bacterial strain were obtained from culture bank of QOL, UVAS Lahore, Pakistan.

Production of Immune sera

Monovalent and trivalent Foot and Mouth Disease Virus vaccines were prepared by using 70% montanide oil and 30% FMD virus ($TCID_{50}$ 10^6). FMDV monovalent vaccine strains O, A, and Asia1.1 ml ($TCID_{50}$ 10^6) were injected intramuscularly in rabbits, separately. FMDV O, Asia 1 and A strains were combined as trivalent vaccine and were injected in other rabbit, while one rabbit was kept uninnoculated control. Antibodies in the sera were detected by using agar gel precipitation test [12].

Culturing and processing of S. aureus

Staphylococcus aureus (obtained from QOL, UVAS, Pakistan) was cultured in brain heart infusion broth. Catalase and coagulase tests were performed for *S.*

Table 1: Optimization of COAT reagents

FMD antigen	Dilution of *S. aureus* and quantity of serum					
	1/10 + 125		1/100 + 250		1/200 + 500	
	Reaction with nonspecific antigen	Reaction with specific antigen	Reaction with nonspecific antigen	Reaction with specific antigen	Reaction with nonspecific antigen	Reaction with specific antigen
Asia1	+	+	_	+	_	_
A	+	+	_	+	_	_
O	+	+	_	+	_	_

Table 2: Reaction of conjugates with FMD Asia1, A and O strains

FMD antigen used	Conjugates				
	Anti-Asia1 conjugate	Anti-A conjugate	Anti-O conjugate	*Combined conjugate	*Control conjugate
serotype Asia1	+	-	-	+	-
serotype A	-	+	-	+	-
serotype O	-	-	+	+	-

*Control conjugate: prepared by using serum from control rabbit; *Combined conjugate: prepared by using anti-Asia1, anti-A and anti-O sera

aureus and grown on Mannitol Salt Agar (MSA) using the standard procedures [13, 14].

Tryptic soy broth was used for secondary culturing of *S. aureus*. Following 24 hours incubation at 37°C the culture was centrifuged for 30 minutes at 3500g. S. *aureus* pellet was washed with Phosphate Buffer Saline (PBS) (0.03M phosphate, 0.12M NaCl, pH 7.3) and inactivated with 0.5% formalin solution for 3hour at room temperature. Inactivated *S. aureus* was washed 3 times with PBS by centrifugation. *S. aureus* was diluted 10% in 0.02% PBS containing sodium azide and kept at 4ºC. The Optical Density (OD) value of *S. aureus* suspension was determined at 525nm by spectrophotometer [15].

Conjugate preparation

Each of the monovalent rabbit anti-O, anti-Asia1, anti-A and trivalent serum (anti-O, anti-Asia1 and anti-A) and Control sera was taken in three different quantities 125µl, 250µl and 500µl. These dilutions were mixed with 2ml of *Staphylococcus aureus* suspensions (1/10, 1/100 and 1/200 dilutions, respectively) and kept at 37°C for 1.5 hour then centrifuged for 15min. at 3500g. Pellet was washed twice by centrifugation with 0.02% sodium azide containing PBS. The conjugate was suspended in 2ml PBS with 0.02% Sodium azide and 0.25% Tween20 then kept at 4°C.

Coagglutination test

COAT reagents were tested against Lab FMD O, Asia1 and O virus and also against cell culture media, phosphate buffer saline, healthy animal saliva and PPR virus. Forty samples of vesicular fluid (VF) were colleted from FMD clinically suspected animals. All the VF samples were tested with COAT for detection of FMD virus. Healthy animal saliva was used as a negative control. Laboratory FMD vaccine strains were used as a negative control. Vesicular fluid collected from FMD infected cow was serially diluted two fold in PBS up to 1:128 and tested with COAT. Sensitivity was determined by the formula: number of true positive results divided by the number of true positive and false negative results [16].

Results

Serum samples collected from vaccinated rabbits were found positive and uninoculated control was found negative against FMD antigens. *S. aureus* produced yellow colony with yellow zones on Pinkish red MSA plate and also found catalase and coagulase positive.

The OD values of *S. aureus* in 1/10, 1/100 and 1/200 dilutions were 1.925, 0.574 and 0.351, respectively. COAT reagents prepared by using *S. aureus* 1/10 dilution yielded non-specific reaction.

Table 3: Reaction of conjugates with cell culture media, normal animal saliva and buffer

Conjugates	Buffer	PPR virus	Cell culture media	Normal animal saliva
Anti-Asia1 conjugate	–	–	–	–
Anti-A conjugate	–	–	–	–
Anti-O conjugate	–	–	–	–
Combined conjugate	–	–	–	–
Control conjugate	–	–	–	–

Table 4: Reaction of combined conjugate (Prepared by using trivalent sera) with 40 field vesicular fluid samples

Samples #	Foot and mouth disease virus serotypes					
	Combined conjugate	Asia1	A	O	sensitivity	Cumulative values of Asia1, A and O
*10	9	0	1	8	100%	9
**26	25	2	1	22	100%	25
***4	4	1	0	3	100%	4
Total 40	38	3	2	33	100%	38

*Vesicular fluid samples collected from Sharaq pur area of Punjab; ** Vesicular fluid samples collected from D.I.Khan; *** Vesicular fluid samples collected from Jhang

No reaction was observed with 1/200 dilution. Whereas, the conjugate containing 250µl immune serum and 1/100 *S. aureus* suspension was found reactive for laboratory FMD O, Asia1 and A types (Table 1).

COAT applied on laboratory FMD O, Asia1 and A antigen was found positive by using prepared anti-O, Anti-Asia1 and Anti-A conjugate, respectively. COAT also applied on FMD O, Asia1 and A antigen by using combined conjugate (prepared by using FMD O, Asia1 and A immune sera) was also found positive (Table 2). Coagglutination test performed on phosphate buffer saline, PPR virus, BHK 21 cell culture media and healthy animal saliva by using FMD anti-Asia1, anti-A, anti-O conjugate, combined conjugate (anti- O, anti-Asia1 and anti-A serum) and control conjugate. No coagglutination reaction was observed and COAT was found highly specific (Table 3). A total 40 field samples were tested with COAT. Thirty-eight out of 40 were found positive by using combined conjugate. Out of 38 vesicular fluid samples typed, 33 of them were O, 3 were Asia1 and 2 were A strains of FMD. According to formula sensitivity was 100% (Table 4). COAT yielded positive results up to 1:32 dilution of VF dilutions (Table 5).

Discussion

Rapid diagnosis of foot and mouth disease is necesssery to control the disease. In present study coagglutination test was standardized for detection of FMD virus in vesicular fluid samples. For preparation of COAT reagents immune sera agianst FMD virus was produced in rabbits. Protein A of Staphylococcus react well with IgG of pig, rabbits and guine pig and less with mouse and bovine IgG [17].

In the present study, *Staphylococcus aureus* was cultured in tryptic soy broth after that further procesing was carried out. Some researchers used brain heart infusion for culturing of *S. aureus* [18]. Some other researchers also used tryptic soy broth for culturing [19]. Culturing of *S. aureus* in tryptic soya broth give better results than culturing in brain heart infusion.

COAT was applied on known antigen of foot and mouth disease virus and generated clear coagglutination within 5 minutes. In preparation of COAT reagents 1/10, 1/100 and 1/200 dilutions of *S. aureus* were used. COAT reagents prepared by using 1/100 dilution of *S. aureus* and 250ul anti-FMD immune sera give specific and sensitive results. Non-specific reaction was yielded by 1/10 dilution and no reaction was observed by 1/200 dilutions. The results of this study are in accordance with other investigators who also reported positive result by testing equal quantity of COAT reagent (1/100 dilution of *Staph. aureus* and 250ul anti-FMD sera) and FMD antigen [19]. Forty vesicular fluid samples were tested with COAT. Thirty-eight VF samples were found positive and 2 VF samples yield negative

Table 5: Sensitivity of Coagglutination test by using combined conjugate against two fold dilution of Vesicular fluid collected from FMD infected animal

	Vesicular fluid two fold dilutions							
Conjugate	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
*Combined conjugate	+	+	+	+	+	-	-	-
Anti-Asia1 conjugate	+	+	+	+	-	-	-	-
Anti-A conjugate	+	+	+	+	-	-	-	-
Anti-O conjugate	+	+	+	+	+	-	-	-

*Combined conjugate: prepared by using trivalent (FMD Asia1, A and O) sera.

Table 6: Vesicular Fluid two fold Dilution

	Vesicular fluid two fold dilutions							
Conjugate	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
*Combined conjugate	+	+	+	+	+	-	-	-
Anti-Asia1 conjugate	+	+	+	+	-	-	-	-
Anti-A conjugate	+	+	+	+	-	-	-	-
Anti-O conjugate	+	+	+	+	+	-	-	-

results. COAT detected FMD virus in vesicular fluid samples. In this study 33 cattle vesicular fluid samples were typed, as O, and 3 were Asia1 and 2 were A serotype of FMDV with COAT. COAT was found 100% sensitive. When tested for specificity, coagglutination test provided positive results only with FMD antigen and did not cross-react with any of the other viral antigens and found highly specific. These findings are in line with results of another study (19). Higher Specificity and sensitivity of the test may be due to similarity of FMD vaccine strain matching with the field virus. All the serum samples were checked qualitatively on Agar Gel Precipitation test.

Conclusions

Result of the present studies, indicated that Coagglutination test can be used succsesfully for detection of foot and mouth disease antigen in vesicular fluid samples. The aim of the study was to apply COAT directly on vesicular fluid samples without culturing on cell culture media. Similar kind of studies have investigated before (20-22), however, here COAT has also been used for detection of Newcastle disease and avian leucosis, for typing of pneumococci, detection of brucella antigen, differentiation of mycoplasma and goat pox antigen, for detection of parvovirus in dog, detection of Rota virus (17-19).

Acknowledgement

The authors are highly thankful to Prof. Dr. Khushi Muhammad Chairman Department of Microbiology and Director Quality Operation Lab to provide cultures from culture bank and facilities of experimental work.

References

[1] Pereira HG. Foot-and-mouth disease. *In*: Virus Diseases of Food Animals. Gibbs EPJ (Ed.): Vol. 2, Academic Press Inc., New York. 1981, p. 333-363.
[2] Sanger DV. The replication of picornaviruses. J Gen Virol 1979; 45: 1-13.
[3] Afzal H, Ellahi A. A study of foot and mouth disease in Pakistan. Bull Int Epizoo 1966; 65: 101-10.
[4] Lewis RN, Mcclellan DA, Crandall KA. The evolution of foot-and mouth disease virus: pacts of recombination and selection. Inf. Genet Evo 2008; 8: 786-798.

[5] Office International des Epizooties/World Organisation for Animal Health. Foot-and-mouth disease. *In*: Terrestrial animal health code, 16th edi. ch. 2.2.10 and appendix 3.8.7. Paris, France, 2009.
[6] Ajmal M, Arshad M, Ahmad MD. Coordinated Research entitled Epidemiology of Major Livestock Diseases in Pakistan (Pilot Study). 5th Annual Report, Pakistan Agri Res Council, Islamabad, Pakistan, 1989.
[7] Zulfiqar M. Draft report for development of national disease control policy for foot and mouth disease in Pakistan under the FAO Project "Support for Emergency Prevention and Control of Main Transboundary Animal Diseases in Pakistan Rinderpest, FMD, PPR". 2003.
[8] Ferris NP, Dawson M. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular disease. Vet Microbiol 1988; 16: 201-209.
[9] Muhammad I, Hanif A, Ahmad MD, Najeeb I, Mahmood A, Aslam HB, Qasim M, Shahzad KA, Ahad A. Development and optimization of multiplex PCR for the identification of O, A and Asia-1 serotypes of FMDV in Pakistan. J Inf Mol Biol 2014; 2 (1): 11–15.
[10] Kessler SW. Use of protein A-bearing staphylococci for the immunoprecipitation and isolation of antigens from cells. Meth Enzymol 1981; 73: 442-458.
[11] Cullen GA, Wyeth PJ. Quantitation of the antibodies to infectious bursal disease. Vet Rec 1975; 97: 115.
[12] Shakya S, Joshi RK. Coagglutination test for rapid detection of infectious bursal disease virus antigen. Trop Ani Hlth Prod 1997; 29: 105-107.
[13] Lui MDLM, Marie TP, Ellen JB. *In:* Color Atlas of Microbiology. Publisher Don ladig. Mosbay- Year book, Inc. 1997; 2: 32-33.
[14] Gilligan PH, Kiska DL, Appleman MD. Cumitech 43: Cystic Fibrosis Microbiology. Coordinating ed., M. D. Appleman. ASM Press, Washington, D.C. 2006.
[15] Montassier HJ, Junior JPA, Pinto AA. Rapid coagglutination test for the detection and typing of Foot and Mouth Disease Virus. J Virol Meth 1994; 50(1-3): 29-41.
[16] Forsgren A, Sjoquist J. Protein A from S. *aureus*. I. Pseudo-immune reaction with human -y globulin. J Immunol 1966; 97: 822-827.
[17] Morsy MA, Panangala VS, Greha MM, Kinnucan MT. A coagglutination assay with monoelonai antibodies for rapid laboratory identification of *Mycoplasma synoviae*. Avi Dis 1992; 36:149-153.
[18] Unver G, Alkan F. Serotyping of FMDV with coagglutination test as an alternative to CFT and ELISA. Report of the session of the FAO research group of the standing technical committee of the European Commission for the control of foot-and-mouth disease Borovets, Bulgaria. 2000; 9:152-158.
[19] Mathur MS, Bhave GG Comparative evaluation of coagglutination and latex agglutination test (Rotalex kit) for detection of rota virus. 1993; 39: 130-131.
[20] Muhammad Hassan Mushtaq, Irfan Khattak, Nisarul Haqb, Furqan Awan. Mass Vaccination and Surveillance Reduced the Burden of Foot and Mouth Disease. Veterinaria; March, 2014; 2(2): 1-5.
[21] Iqbal MN, Muhammad A, Anjum AA, Shahzad KA, Ali MA, Ali S. Epidemiology of Gigantocotyle explanatunin naturally infected buffaloes. Veterinaria 2014; 2(1): 15-18.
[22] Muhammad Younis, Muhammad Iqbal, Neelma Shoukat, Beenish Nawaz, Feroza Hamid Wattoo, Khawar Ali Shahzad. Effect of chemotherapy and radiotherapy on red blood cells and haemoglobin in cancer patients.. Sci. Lett.; March, 2014; 2(1): 15-18