EVALUATION OF AN INDIRECT ELISA FOR DETECTION AND TYPING OF FOOT-AND-MOUTH DISEASE VIRUS

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Abstract

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An Indirect enzyme linked immunosorbent assay (ELISA) kit was used for diagnosis of foot-and-mouth disease virus (FMDV) types O1, A24, C3 which occurred in Rio Grande do Sul State, Southern Brazil during 1984-1994. The samples were randomly selected and tested by ELISA, Complement Fixation Test (CFT) and in tissue culture. Out of 106 samples 78 (73,5%) were positive by ELISA and 39 (36,8 %) were found positive in CFT, when original suspensions were used. Once these samples were inoculated onto tissue culture both tests gave similar results, although ELISA picked up more positive samples during the 1st passage in tissue culture. The negative samples (16) included in this study were negative in all tests. The ELISA was more sensitive than and as specific as CFT. ELISA and tissue culture together were shown to be a better system for detection of foot-and-mouth disease virus antigen than CFT.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and is one of the most important viral disease affecting livestock. Its major effect is as a constraint to international trade (export/import) between FMD-free countries and those in which FMD is endemic (South America, Africa and Asia). As Rio Grande do Sul State, Brazil, is involved in the Programme of Control and Eradication of FMD in the River Plate Basin Area, involving Argentina, Brazil, Paraguay and Uruguay [1] it is very important to have early notification of outbreaks and rapid diagnosis. Any failure in diagnosis will affect disease control and will favor the spread of infection. All strategies of control and eradication of FMD in this area rely on effective vaccination of cattle, a network of veterinary officers and efficient diagnostic laboratories. Thus, it is necessary that laboratory tests for FMD should have very good sensitivity, specificity and reliability.

In most countries of South America detection and typing of FMDV has been carried out by CFT [2,3], however, CFT has many disadvantages such as: low sensitivity, it is cumbersome, time consuming, and requires a good laboratory structure. The advantages of indirect ELISA for typing of FMDV have been described [4,5,6,7,]. Thus, the purpose of this investigation was to evaluate an ELISA kit for FMD antigen detection and compare it with the CFT and tissue culture, using the virus collection of IPVDF's FMD Unit and samples submitted from outbreaks, at IPVDF-Regional Diagnosis Laboratory, Rio Grande do Sul State.

2. MATERIAL AND METHODS

2.1. Field Samples

Epithelial samples collected from 1984 up to 1994 were sent to IPVDF, Regional Diagnosis Laboratory. A total of 90 positive samples (Type O1: 25; type A24: 46; type C3: 19) and 16 known negative samples were stored at -20°C in PBS, pH 7.4 50 % glycerol. All samples were tested by ELISA, CFT and inoculated onto tissue culture (roller bottles) either as original suspensions or as tissue culture supernatants.

2.2. CFT

CFT used was a tube test (CF 50%) standardized by the Panamerican Foot-and-Mouth Disease Center (PAFMDC) for FMDV [2].

2.3. ELISA procedure

An ELISA kit provided by the Joint FAO/IAEA Division , Vienna, Austria was used. It is based on an indirect sandwich ELISA. Briefly, rabbit antisera specific for the different types and subtypes of FMDV and Vesicular Stomatitis Virus (VSV) are adsorbed to polystyrene plates. Following the addition of the test sample, the antigen is trapped by the immobilized antibodies. Specific guinea pig antisera are added to react with the trapped antigen. The reaction is detected by the addition of anti-guinea pig antibody conjugated to horseradish peroxidase (HRP). After the addition of substrate/chromogen a colored reaction develops allowing identification of the antigen [8].

2.4 Virus Isolation

One ml of the original suspensions were inoculated onto cultures of IBRS-2 cells grown in 1 liter disposable plastic bottles. The monolayers were washed with 50 ml of maintenance medium and subsequently 100 ml of the same medium was added. After inoculation these bottles were incubated at 37°C in roller apparatus for 48 hours or harvested earlier if cytopathic effect was observed.

3. RESULTS

The results obtained by ELISA and CFT with original suspensions are shown in Table I. ELISA was positive in 73.5% while 36.8% were positive by CFT. All samples(positives and negatives) were inoculated onto tissue culture(three passages) and results are shown in Table II. Both tests successfully detected virus in the tissue culture supernatants but ELISA identified more positive results than CFT at the 1st passage. In these cases the samples were inoculated (2nd and 3rd passages) to increase the virus titer and subsequently CFT gave a positive typing. ELISA was not able to detect FMDV in 28 original suspensions(26.5%) and CFT failed to detect virus in 67 original suspensions(63.2%). The results with negative samples(16), included in this experiment, had complete agreement in all tests. The sensitivity and specificity of ELISA and CFT are shown in Tables III and IV.

TABLE I. TYPING OF FMDV BY ELISA AND CFT USING ORIGINAL SUSPENSIONS OF FIELD SAMPLES

	CFT	ELISA
Positive	39(36.8%)	78(73.5%)
Negative	67(63.2%)	28(26.5%)
Total	106	106

TABLE II. TYPING OF FMDV BY ELISA AND CFT ON CELL CULTURE HARVESTS

		1st passage	2nd passage	3rd passage
CFT	Positive	79(75.5%)	90(85.0%)	
	Negative	27(25.5%)	16(15.5%)	16
ELISA	Positive	90(85.0%)		
	Negative	16(15.0%)	16	16

TABLE III. SENSITIVITY AND SPECIFICITY OF ELISA FOR DETECTING FMDV IN EPITHELIAL SAMPLES (ORIGINAL SUSPENSIONS)

		Positive	Negative	Total
ELISA	Positive	78	0	78
	Negative	12	16	28
	Total	90	16	106

Sensitivity: 86.6% / Specificity: 100%

TABLE IV. SENSITIVITY AND SPECIFICITY OF CFT FOR DETECTING FMDV IN EPITHELIAL SAMPLE S (ORIGINAL SUSPENSIONS)

		Positive	Negative	Total
CFT	Positive	39	0	39
	Negative	51	16	67
	Total	90	16	106

Sensitivity: 43.3% / Specificity: 100%

4. DISCUSSION

CFT has been used in South America as the standard test for diagnosis of FMD and other vesicular diseases since 1960 and recommended by the PAFMDC to be used at diagnostic laboratories in all countries in this continent [2]. Since ELISA has been shown to be a sensitive test for diagnosis of FMD [2,3,4,5,6,7,9,10] it is now in use in a majority of laboratories throughout the world for antigen and antibody detection. In this study it was possible to confirm once more the disadvantages of CFT in relation to ELISA (Tables I - IV) for detection and typing of FMD. One disadvantage of the ELISA Kit was the short shelf life of the reconstituted positive controls. Once diluted they kept acceptable activity for no more than three months as an average between the two batches received for this investigation. It is an aspect that will need additional studies with diluents that may improve antigen stability. Cross reactions were not a problem. When they occurred (four tissue culture samples O/C) they were probably due to high antigen content, since it was not detected when original suspensions were typed. Also in this study it was possible to show (Table II) that ELISA and tissue culture were the best system for the detection of FMDV.

As ELISA proved to be simple to perform, rapid and has high sensitivity it will be very useful for FMD control and diagnosis in countries or areas such as the River Plate Basin Programme where disease needs to be confirmed as soon as possible in order to prevent spread and involvement of new areas.

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REFERENCES

[1] COSALFA- Comisión de la Lucha Contra la Fiebre Aftosa, Informe Final XX Reunión Ordinaria, Montevideo, Março, (1993) p.25-26.

[2] ALONSO, A., Diagnóstico de las Enfermedades Vesiculares, Rio de Janeiro: CPFA, 1986. 40p. (Series de Manuales Técnicos, 15).

[3] ALONSO, A.F., MARTINS, M..A., GOMES, M.P.D., ALLENDE, R., SÖNDAHL, M.S. Foot-and-Mouth Disease virus typing by complement fixation and ELISA tests using monovalent and polyvalent antisera, J. Vet. Diagn. Invest. **3** (1992) 287-292.

[4] HAMBLIN, C., ARMSTRONG, R. M., HEDGER, R.S., A rapid enzyme-linked immunosorbent assay for the detection of foot-and-mouth disease virus in epithelial tissues, Veterinary Microbiology, Netherlands, 9 (1987) 435-443.

[5] FERRIS, N.P., DOWSON, M., Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular disease, Veterinary Microbiology, Netherlands **16** (1988) 201-209.

[6] ROEDER, P. L., Le BLANC SMITH, P. M., Detection and typing of FMD virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis, Research in Veterinary Science, USA, **43** (1987) 225-232.

[7] KITCHING, R.P., Current status for the standardization and supply of reagents for FAO/IAEA FMD ELISA kits. In: IMMUNOASSAY METHODS FOR THE DIAGNOSIS AND EPIDEMIOLOGY OF ANIMAL DISEASES IN LATIN AMERICA, Bogota, Colombia, Proceedings of the 2nd Research Coordination Meeting FAO/IAEA/SIDA 1992, FAO/IAEA, 4-5.

[8] FAO/IAEA, FMD ELISA kit manual, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Austria (1992).

[9] ABU-ELZEIN, E., CROWTHER, J., Enzyme-labeled immunosorbent assay techniques in foot-and-mouth disease virus research, J.Hyg. **80** (1978) 391-400.

[10] CROWTHER, J.R., ABU-EL ZEIN, E.M.E., Detection and quantification of foot-and-mouth disease virus by enzyme-labelled immunosorbent assay techniques, Journal of General Virology, London, **42** (1979) 597-602.