SEÇÃO: VETERINÁRIA

AVALIAÇÃO DE UM TESTE DE ELISA INDIRETO PARA DETECTAR E TIPIFICAR VIRUS DA FEBRE AFTOSA

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RESUMO - Um kit de elisa indireto foi usado para diagnosticar o virus da febre aftosa para os tipos O1, A24, C3 incidentes no Estado do Rio Grande do Sul no período 1984 - 1993. As amostras foram selecionadas ao acaso e testadas por elisa, fixação do complemento(FC) e em cultura de tecido.De um total de 106 amostras de suspensões originais, 78(73,5%) foram positivas em elisa e 39(36,8%) foram positivas por fixação do complemento. Quando estas amostras foram inoculadas em culturas de células, ambos os testes tiveram idêntica performance, embora o teste de elisa tenha sido capaz de detectar mais amostras positivas que o teste de fixação de complemento durante a 1ª passagem em cultura de tecido. Todas as amostras negativas(16) incluídas no experimento se mostraram negativas em todos os testes e durante toda a investigação. O teste de elisa foi mais sensível que FC; elisa e FC tiveram a mesma especificidade. Elisa e cultura de tecidos se mostraram o melhor sistema para detectar o antígeno de febre aftosa que o teste de FC.

Palavras-chave: febre aftosa; elisa indireto, fixação de complemento, cultura de tecidos.

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

ABSTRACT - A Indirect enzyme-linked immunosorbent assay (ELISA) kit was used for diagnosis of foot and mouth disease virus (FMDV) types O1, A24, C3 which occured in Rio Grande do Sul State, Southern Brazil during 1984-1993. 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 those 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 (FMD) antigen than CFT.

Key words: Foot and Mouth Disease; Indirect Elisa; Complement Fixation;Tissue Culture.

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INTRODUCTION

Foot and Mouth Diseases (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 Programm of Control and Eradication of FMD in the River Plate Basin Area, involving Argentina, Brazil, Paraguay and Uruguay(COSALFA,1993) it is very important to have early notification of outbreaks and rapid diagnosis. Any failure in diagnosis will affect disease control and will favour 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 by CFT(ALONSO,1986) , however, CFT has many disadvantages such as: low sensitivity, it is cumbersome, time consumming, and requires a good laboratory structure to set up the technique. The advantages of indirect Elisa for typing of FMDV have been described (HAMBLIN et al.,1984;FERRIS and DOWSON,1988; GOMES et al.,1989; ROEDER and LEBLANC SMITH,1987; KITCHING,1992). Thus, the purpose of this investigation was to evaluate an Elisa Kit for FMD antigen detection and compare it with 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.

MATERIAL AND METHODS

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;type C3:19) and 16 known negative samples were stored at -20° C in PBS, pH 7.4 with 50 % glicerol. All samples were tested by ELISA, CFT and inoculated onto tissue culture (roller bottles) either as original suspensions or as tissue culture supernatants.

CF Test

The CF test used was a tube test (CF 50%) standardized by the Panamerican Foot and Mouth Disease Centre (PAFMDC) for FMDV(ALONSO, 1986).

Elisa procedure

Elisa Kit provided by Joint Division FAO/IAEA-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 antiguinea pig antibody conjugated to HRP. After the addition of substrate/chromogen a coloured reaction develops allowing identification of the antigen(JOINT FAO/IAEA Elisa Manual, v.1.1, 1992).

Virus Isolation

One ml of the original suspensions were inoculated into cultures of IBRS-2 cells grown in 1 litre disposable plastics 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 37C in roller apparatus for 48 hs or harvested earlier if citopathic effect was observed.

RESULTS AND DISCUSSION

The results obtained by Elisa and CFT with original suspensions are shown in Table 1. 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 2. Both tests succefully 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 titre 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 complet agreement in all tests. The sensitivity and specificity of Elisa and CFT are shown in Tables 3 and 4.

TABLE 1 – 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 2 – 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 3 – Sensitivity and Specificity of Elisa for detecting FMDV in epithelial samples(original suspensions)

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

Sensitivity:86.6% - Specificity: 100%

TABLE 4 – Sensitivity and Specificity of CFT for detecting FMDV in epithelial samples(original suspensions)

		Positive	*Negative*	*Total*
CFT	Positive	39	0	9
	Negative	51	16	67
	Total	90	16	106

Sensitivity: 43.3% - Specificity: 100%

CFT has been used in South America as the standard test for diagnosis of FMD and other vesicular diseases since 1960 and reccomended by the PAFMDC to be used at diagnostic laboratories in all countries in this continent(ALONSO,1986). Since Elisa has been shown to be a sensitive test for diagnosis of FMD(ABU-ELZEIN and CROWTHER,1978; CROWTHER and ABU-ELZEIN, 1979) it is now in use in a majority of laboratories throughout the world for antigen and antibody detection. In this study was possible to confirm once more the disadvantages of CFT in relation to Elisa(Tables 1,2,3 and 4) for detection and typing of FMD. One disadvantage of Elisa Kit tested was the short shelf life of the reconstituted Positive Controls(inactivated antigens). 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 contents since it was not detected when original suspensions were typed.

CONCLUSIONS

Elisa is simple to perform, rapid and with high sensitivity it has good application in FMD control and diagnosis in any country or areas under erradication programms.

Elisa and tissue culture showed to be the best system for detection and diagnosis of FMD virus.

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