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WESTERN BLOT IN COMPARISON WITH ELISA FOR DETECTING ANTIBODIES AGAINST FOOT AND MOUTH DISEASE VIRUS

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SUMMARY

Western blot technique is described for visual detection of foot and mouth disease virus (FMDV) antibodies in sera of infected, vaccinated and field cattle using FMDV fragments separated by SDS-PAGE and blotting onto nitrocellulose membrane. The interaction between FMDV antibodies and blotting virus fragments revealed dark blue bands, which is considered a positive result by Western blot. All sera of infected, vaccinated bulls and 10 out of 38 field animals had antibody titers ranged from 1.7 to 3.1 by ELISA. All ELISA positive sera demonstrated dark blue bands against the different FMDV fragment by Western blot. The use of Western blot as a rapid specific and sensitive technique for detecting FMDV antibodies was discussed.

Keywords: Western blot, ELISA, serotype O1/93 FMDV antibodies, infected, vaccinated and fields cattle.

INTRODUCTION

Foot and mouth disease virus (FMDV) is a member of Picornaviridae family, it has a single stranded positive sense RNA molecule of about 8.2 kilo base. The fragments of the virus contains four structural protein (1A, 1B, 1C &1D) of the virus capsid and eight non-structural protein (NS) proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D & L protein) involved in the life cycle of the virus inside the infected cells (Kitching 2002). The sera of infected animals contain both structural and non-structural fragments while the sera of vaccinated animals contain structural fragment (Mackay et al., 1998). Western blot technique has been applied to study the immune reactions of a range of virus proteins (Towbin et al., 1970). On the base of Enzyme Linked Immunosorbent assay (ELISA), protein fragments of the virus separated by SDS-PAGE and transferred onto nitrocellulose membrane can be reacted with a specific primary

antibody. The interaction between blotting fragment of the virus and the tested sera indicate the presence of antibodies against the separated fragment of the virus. Loh et al., 1985 and Afshar et al., 1986 reported that Western blot is specific and efficiently than ELISA. Immunoreactivity in Western blots does not necessarily detect the possible neutralizing epitopes in the viral proteins (Kamstrup et al., 1991). To examine the applicability of Western blot for detecting antibodies against FMDV, the tested sera were evaluated firstly by ELISA as a rapid and sensitive method for detecting antibodies against FMDV. The aim of the present study is directed to apply Western blot to be used as a rapid and sensitive technique for detecting FMDV antibodies in field animals which could facilitates the control of the disease in Egypt.

MATERIAL AND METHODS

Serum samples

A total of 50 serum samples were collected from eight vaccinated, four infected bulls of one year old and 38 field serum samples collected from cattle of 1.5 to 2 years raised at Sharqiea Governorate. The sera were inactivated at 56°C for 30 minutes and kept at -20°C until used.

Antigen production

Virus Purification and concentration was carried out according to the methods described by (Killington et al., 1996). Serotype O1/93 foot and mouth disease virus propagated in BHK-21 clones 13 was used. Removal of lipid and cell debris was carried out using chloroform at a concentration of 1.5% and centrifuged at 3000 rpm for 30 minutes in cooling centrifuge. PEG 6000 (Fluka chemie GmbH; No. 81260) was added to the clear virus with stirring to 7% (w/v) final concentration at 4°C for 2 hours then overnight. The mixture was centrifuged at 6000 r.p.m. for 30 minutes. The precipitate was resuspended to a final concentration of 1% of the original volume in elution buffer of pH 7.6 (2.42g TRIS, 22.5g KCL in 1L de-mineralized - water) to elute the virus from PEG6000. harvest the supernatant and kept at -20°C or -70°C until used.

Western blot.

SDS-PAGE electrophoresis was performed using 10% polyacrylamide gel as described by (Towbin et al., 1970), with modification described by Knowles & Hedger (1985). One volume of eluted serotype O1/93 FMD virus suspended in 5 volume of SDS-PAGE sample buffer (10 mM Tris-Hcl pH 6.8, 25% vol/vol glycerol, 10% wt/vol SDS, 0.02% wt/vol bromophenol blue, 10% β- mercaptoethanol). The mixtures boiled for 3 min and separated by SDS-PAGE using a 10% polyacrylamide gel. After electrophoresis one gel contains separated bands of the virus and low molecular weight protein marker (18, 30, 45, 66 and 98 KDPharmacia) was stained with Coomassie Brilliant blue (0.006% Coomassie brilliant blue G250 wt/v). The other gels, the separated bands were transferred from the gel to

nitrocellulose membranes (0.2µm) using 40mM glycine, 50mM Tris, 1.3mM SDS, 20% v/v methanol as buffer. Transfer was performed with 200 mA for 2 h at room temperature. Immune reactions after blotting of viral proteins were carried out with the following steps: Blocking with Tris buffered saline (Tris 50 mM, 200 mM NaCl and "Tween 80" 0.05%) containing 3% bovine albumin for 1 h. The incubation with tested sera and antibovine conjugate (conjugated with horseradish peroxidase enzyme) was carried out as described by (Shawky et al., 2000). The specific antigen antibody reaction was visually detected using a solution of substrate (3mg of 4-chloro-1-naphthol/ml/absolute ethanol activated with H_2O_2 (3%). Specific positive reactions appeared as a dark blue band at the individual site of the separated proteins after incubation at room temperature for 15 minutes and wash of the membrane with distilled water. The positive results were photographed.

Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA):

The procedure was carried out as described by Hamblin et al., (1986) and Shawky et al., (2000). The optimum dilution of the antigen used for coating was 1/160, bovine conjugate 1/10.000 and the sera were tested in a fixed dilution of 1/10.

RESULTS

Western blot from concentrated and purified serotype O1/93 FMD virus showed at least 3 distinct bands with apparent molecular masses of 98 kD, 45 kD and 30 kD (Fig.1). Antibody titers against serotype O1/93 foot and mouth disease virus detected by ELISA in the sera of infected, vaccinated bulls and field animals are shown in table 1. All sera of infected, vaccinated and 10 out of 38 field sera had antibody titers ranged from 1.75 to 3.1 by ELISA (table 1). Comparative evaluation of sensitivity of ELISA and Western blot for detecting antibodies against FMDV illustrated in table 2. Specific positive reaction of the tested sera appeared as a dark blue band at the individual site of the separated fragments of serotype O1/93 FMDV. Fig.2.

Table 1.Positive antibody titers detected by ELISA in sera of infected, vaccinated and field cattle.

Animals	Positive antibody titers detected by ELISA										Positive %
	Animal number										
	1	2	3	4	5	6	7	8	9	10	
Vaccinated	1.7	2.2	3.1	1.7	2.2	2.0	3.1	2.2	-	-	8/8 (100%)
Infected	2.2	3.1	3.0	2.2	-	-	-	-	-	-	4/4 (100%)
Field animals	1.7	1.8	2.0	1.8	2.2	1.7	1.8	2.2	2.0	1.7	10/38 (26%)

Table 2. Comparative evaluation the sensitivity of Western blot by ELISA for detecting antibodies against FMDV

Assay	Antibodies detected in sera tested by ELISA and Western blot											
	Total No. of sera tested			Positive %			Negative %			Positive %		
	Inf.	Vac.	Fld	Inf.	Vac.	Fld.	Inf.	Vac.	Fld	Inf.	Vac.	Fld
ELSA	4	8	38	4*	8*	10*	0	0	28	100%	100%	26%
Western blot	4	8	38	4**	8**	10**	0	0	28	100%	100%	26%

Inf. Infected animals. Vac. Vaccinated animals. Fld. Field animals.

* Positive antibody titers ranged between 1.95 to 3.2. By ELISA

** Positive results appeared as a light brown band at the individual site of the separated fragments of serotype O1/93 FMDV.

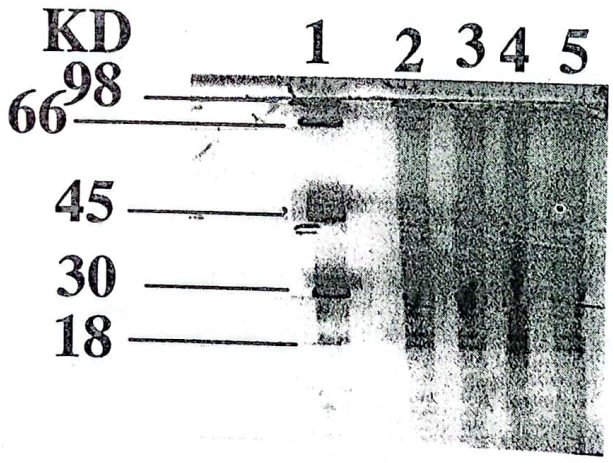

Fig. 1. Western blots of concentrated and purified serotype O1/93 FMD virus. Three bands of molecular weight 98kD, 45kD and 30kD are marked by arrows.

Lane 1 :protein marker (18kD- 98kD) **Lanes 2,3,4&5:** fragments of FMDV

Fig. 2. Western blot analysis showing dark blue bands detected in the positive sera of infected vaccinated and field animals

DISCUSSION

In this study Western blot technique using foot and mouth disease virus (FMDV) fragments separated by SDS-PAGE and blotting onto nitrocellulose membrane has been developed for visual detection the immune reactions of FMDV antibodies in serum samples providing a convenient, time-saving and an objective evaluation. Because of literatures on Western blot for detecting antibodies against foot and mouth disease virus are fewer the investigated sera firstly tested by ELISA. Based on the positive results obtained by ELISA from sera of infected, vaccinated and field animals, detecting dark blue bands at the individual site of the separated fragments of serotype O1/93 in the same positive ELISA sera by Western blot proves that, Western blot is a diagnostic sensitive and specific test for detecting FMDV antibodies. Our results agree with Neitzert et al., 1991 and Bergmann et al., 1993, they mentioned that, serological testing for antibodies against FMDV using Western blot assay indicated considerable amount of antibodies against replicating antigen in viral infection. The technique is sensitive and specific for detecting antibodies (Sanger & Clark 1986, Neitzert et al., 1991, Bergmann et al., 1993, and Guiso et al., 1993). Western blot could be detected immunogenic protein of FMDV (VP1) in non purified virus and the integrity of VP1 in formulated vaccines (Sanger & Clark 1986). The significant advantages of Western blot was: a) Less antigen required (Guiso et al., 1993). b) More specific and sensitive than

ELISA as nitrocellulose membrane has the ability and affinity to bind proteins more rapid than ELISA plate (Loh et al., 1985 and Afshar et al., 1986). c) Immunoreactivity in Western blot does not necessarily detect the possible neutralizing epitopes in the whole virus like in serum neutralization and ELISA techniques (Kamstrup et al., 1991). The obtained results from field serum samples gave an evidence that serotype O1 could be the only serotype circulated in Egypt. In conclusion a Western blot would be significant addition to the rapid tools available for detecting FMDV antibodies which could facilitates the control of the disease in Egypt.

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