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Production of FMDV ELISA Kit for Antibodies Detection in Animals Using Native Strain With Production of Reference Hyperimmune Serum in Guinea Pigs

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The objective of the present study was to evaluate an enzyme linked immunosorbant assay (ELISA) based kit for detecting foot and mouth disease (FMD) antibodies using a native FMDV strain (O1, Aga 93). Results were compared to those of serum neutralization test (SNT) and 3CD ELISA Kit. Sensitivities of the ELISA Kit and 3CD ELISA were 94% and 91%, respectively. Rapid confirmation of the presence of FMDV antibodies through the developed ELISA Kit may have a significant impact on initiating emergency control of FMD outbreaks.

INTRODUCTION

Foot and mouth disease (FMD) is one of the most important viral diseases of domestic animals. The causative agent is a *Picornavirus* that displays remarkable antigenic variability. Seven distinct serological types and more than 60 subtypes and variants have been identified worldwide (Pereira, 1977). Evidence that carrier animals can transmit the disease to susceptible animals in the field (Hedger and condy, 1985) and experimental transmission of FMD from carrier buffaloes to cattle (Dawe *et al.*, 1994) has been demonstrated. The disease assumes an enzootic form in Egypt causing heavy losses in milk, meat and sometimes death of young animals (Moussa *et al.*, 1984; Daoud *et al.* 1988; and El-Nakashly *et al.*, 1996).

Effectiveness of FMD control measures rely upon the use of rapid, sensitive and reliable diagnostic procedures. Several methods have been employed for the detection of antigens and antibodies following a specific infection. Enzyme linked immunosorbent assay (ELISA) has found its way in this field (Charan and Guatam, 1984) because of its simplicity, sensitivity and specificity over most other serological assays.

At present, ELISA for FMD antigen detection and typing is applied as a routine in most FMD diagnostic laboratories (Alonso *et al.*, 1992).

The purpose of the present work was to develop a local diagnostic ELISA Kit to diagnose FMD and to evaluate the seroconversion in serum samples collected during acute and convalescent stages of infection in cattle and buffaloes.

MATERIAL AND METHODS

Antigens

A- FMDV type O1Aga 93 was used in the SNT assay and for detecting specific antibodies in ELISA.

B- The sequence corresponding to 3 CD region of FMD type O was amplified by reverse transcriptase polymerase chain reaction RT-PCR and cloned into PET 21 a-d vector and induced by Isopropyl-B-D-thio-galaetspyranoside (IPTG) to express the protein antigen of interest (Shawky *et al.*, 1999).

Serum samples

Sixty-six sera collected from cattle and buffaloes during an FMD outbreak in 2000 in Ismaelia and Monofia Governorates, Egypt.

Antisera

Bovine Hyperimmune serum against FMDV type O1 was prepared locally at the FMD department, Serum and Vaccine Research Institute, Cairo. Egypt. Briefly (Graves, 1963), 10 ngm of FMDV was emulsified in complete Freund's adjuvant and inoculated S/C into mature guinea pigs. After 3 weeks, the animals received a booster inoculation with FMDV and the adjuvant. Guinea pigs were bled out after 2 more weeks and antisera were collected and stored at -20oC until used. Evaluation of antisera was conducted by solid phase ELISA using FMDV antigen.

Antigen Titaration

FMDV was titrated against reference positive hyperimmune bovine serum in an ELISA assay. Serial antigen dilutions were made in carbonate bicarbonate buffer (pH 9.6). Immune serum was diluted in phosphate buffer saline (PBS. pH 7.4) containing 2% lactalbumin. Checkerboard titrations were made and optimal antigen titers were determined (OD492 of approximately 1.0) Hamblin *et al.*, (1986).

Detection of FMDV antibodies

SNT was performed in flat bottom culture plates as described by Kisory and Bartha (1972). In ELISA, antibovine horseradish peroxidase conjugate (Sigma) and Anti-guinea pig horse radish peroxidase conjugate (Sigma) were used. Antibody titres were expressed as the logarithm of the reciprocal final dilution of sera neutralizing an estimated 100 tissue culture infective dose 50 (TCID50) at 50% end point. 3CD ELISA was also conducted as described by Shawky *et al.*, (2000).

RESULTS AND DISCUSSION

FMDV antigen titrated against reference positive hyperimmune bovine serum showed a titer of 640 as shown in table 1. Guinea pig Polyclonal antisera titrated against an optimum dilution of FMDV antigen was 1/400 as shown in Table 2. Reciprocal serum dilutions that inhibited 50% of cytopathogenic effects (CPE) caused by 100 TCID 50 of FMDV type O1 were expressed in log10. Table 3 shows the major rise in antibody titers (0.6 log10 – 1.8 log10) between the acute and convalescent stages of the disease.

High antibody titers in a high proportion of convalescent calves were detected at the time of investigation. These results were confirmed by findings from 3CD ELISA. as shown in Table 4.

Table (1) Determination of minimal detectable level of FMDV antigen against optimum dilution of reference hyperimmune serum

ELISA readings								
FMDV Antigen	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Serum reactivity	+	+	+	+	+	+	+	-

Table (2) Evaluation of guinea pig polyclonal antisera against FMDV antigen

Serum dilution*	OD Matrix	
	Inoculated guinea pigs	Control negatives
1/50	1.6	0.4
1/100	1.3	0.3
1/200	1.2	
1/400	1.1	
1/800	0.94	
1/1600	0.6	

*The optimum dilution of reactive guinea pig hyperimmune serum against FMDV antigen was 1/400.

Table (3): FMD antibodies detected in acute and convalescent stages of the disease

Serum neutralization antibody titer			
No. of samples	Acute titer (log_{10})	No. of samples	Convalescent titer (log_{10})
20	0.6 – 0.9	46	1.5 – 1.8

Table (4) Comparative evaluation of sensitivity of 3CD- and antigen ELISA's

ASSAY	No. tested samples	No. positive samples	No. negative samples	Sensitivity
FMDV antigen ELISA	66	62	4	94%
3CD ELISA	66	60	6	91%

In Egypt, control measures for combating FMD depend mainly upon massive vaccination and restriction of animal movement. However, detection of carriers is an important step to control the spread of the disease (Bengis and Eramus, 1988). Lately, FMD has assumed an enzootic form in Egypt where it causes heavy losses in milk, meat and sometimes leads to the death of young animals (El-Nakashly *et al.*, 1996).

Therefore, rapid and accurate diagnosis of FMD infection and carriage among animals is critical for the laboratory detection of FMDV or antibodies. While different techniques are available for their detection, our results show that SNT was suitable for detecting FMD antibodies among infected, vaccinated and possibly

carrier animals. As shown in table (1) these findings are in agreement with Hamblin *et al.*, 1986 and El-Nakashly *et al.*, (1996). However, problems such as low sensitivity of SNT and its long incubation time led to the introduction of other techniques; among them are ELISA and 3CD ELISA (Shawky *et al.*, 2000) for the primary diagnosis of FMD infection (Table 4). Recently a sequence corresponding to 3CD region (non structural genes) of FMDV type O was amplified by RT-PCR and cloned into PET 21- vector. Then it was transformed to expression host, induced by IPTG to express the proteins of the inserted sequence Shawky *et al.*, (1999). The method is applicable for detecting antibodies against FMDV and provided a rapid and accurate means for distinguishing infected from non infected animals. This technique was used as a confirmatory test for the locally developed ELISA kit using a native strain of FMDV type O1 Aga 93.

The whole FMDV antigen was inoculated in guinea pigs to produce polyclonal antibodies. Better antibody response was obtained when Freund's adjuvant as used in 2 immunization doses at an interval (Table 3). These observations agreed with Kerr and Thrope (1994).

The prepared antisera from guinea pigs were compared with control serum from infected bovine using solid phase ELISA as described by Haves and Tensen. (1983). Ferris *et al.*, (1989) and Hamblin *et al.*, (1986).

According to the records of animals (vaccinated vs. exposed), the sensitivity of the ELISA Kit and 3CD ELISA was 94% and 91%, respectively. However, 3CD ELISA detected antibodies against FMDV infection only.

In conclusion, the higher detection rate of the ELISA Kit over SNT and its easy performance, strongly recommends its use for the swift and reliable diagnosis of FMDV.

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