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ELISA AS A RAPID METHOD FOR DETECTING THE CORRELATION BETWEEN THE FIELD ISOLATES OF FOOT AND MOUTH DISEASE VIRUS AND THE CURRENT USED VACCINE STRAIN IN EGYPT

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SUMMARY

Antigenic relationships between recent six serotype O field isolates of foot and mouth disease virus and the current used vaccine strain O1/3/93 can be rapidly determined using one-way liquid-phase blocking sandwich ELISA. The most reliable vaccine strain to control outbreaks caused by field isolates can be rapidly identified using the described relationship "r". All virus isolates shared a closer antigenic relationship to the current used vaccine strain with "r" values ranged between 0.8 and 1. A potent vaccine containing the current used strain O1/3/93 could be the suitable vaccine to protect animals against the existing serotype O field isolates.

Keywords: FMDV type O1 - ELISA - Antigenic relationship - field virus - vaccine strain.

INTRODUCTION

Foot and mouth disease (FMD) serotype O1 was the serotype circulating in Egypt (Daoud et al., 1988 and 2004). Within this serotype there are a large number of strains, which display a spectrum of antigenic characteristics reflecting the external structure of the capsid protein (Kitching et al., 1988). High mutation rate of FMD virus has required the constant monitoring of field strains of FMD virus to ensure that the vaccine in current used is effective (Kitching et al., 1988 &1989, Samuel et al., 1993, Farag et al., 1998). The world reference laboratory for diagnosis of FMD virus (Pirbright, London) is the only laboratory that is able to identify subtype in FMD field virus isolates. To facilitate identification of field strain a system of subtyping was developed and a formula was derived from the relationship between complement fixation titers of guinea pig sera

prepared against field and vaccine strains of FMD virus (Pereira 1978, and Kitching et al., 1989). The ever increasing difficulty of comparing field strains with all other subtypes within the serotype was becoming so time consuming that results were usually only available long after the outbreak caused by the isolate had been controlled (Kitching et al., 1989). Rapid correlation "r" between field isolates and vaccine strain of FMD virus based on a liquid phase blocking sandwich ELISA was reviewed by Kitching et al., (1988 & 1989) and Samuel et al., (1990b & 1993). The authors replacing the conventional subtyping with the rapid correlation "r" and using it to identify suitable vaccine strain that could be able to control virus of outbreak. The relationship "r" is a convenient, time saving, easy, rapid and inexpensive method to be carried out in ordinary FMD laboratory. The aim of this study was to carry out a rapid serological correlation "r" between serotype O field isolates and the current used vaccine strain, and to investigate the protectabililty of the current vaccine to the virus isolates.

MATERIALS AND METHODS

Tongue epithelium samples:

Six tongue epithelium (T.E) samples were collected from 6 bulls of 1 to 1.5 years old from separate outbreaks occurred in three Governorates (Qalubiea, Dakahlyea and Sharqiea provinces). The samples were collected aseptically in 50% glycerin-phosphate buffer pH 7.5 and kept at -20°C until used.

Viruses

Field FMD viruses

Primary isolation of FMD virus from T.E was carried out in tissue-culture monolayers of BHK 21 cells. Viruses were initially typed by complement fixation test as described by Brooksby (1968). All isolates were adapted to grow in BHK 21 cells by six serial passages before being used in a liquid-phase-blocking sandwich ELISA.

Vaccine strain:

The vaccine strain used was O1/3/93 FMDV, isolated from Aga, Dakahlyea since March 1993, typed by CFT as type "O" FMDV. Identification of the virus was confirmed by Foot and Mouth disease World Reference Laboratory (FMD - WRL), Pirbright, London, U.K. The virus was adapted to growth in BHK 21 cells.

Antisera:

Bovine antiserum

Bovine antiserum to FMDV vaccine strain O1/3/93 was used. The serum was collected from vaccinated bull at 21 days post vaccination. The serum was used to estimate the correlation between field isolates and vaccine strain "r".

Rabbit and guinea pig antisera to O1/3/93 FMDV

Antisera to the inactivated 146S antigen of FMDV vaccine strain O1/3/93 were raised in rab-

bit and guinea pig by the method described by Have et al., 1984.

Anti guinea pig conjugate serum

Anti guinea pig serum conjugated with horseradish peroxidase enzyme is supplied by Sigma Company (USA) was used.

A liquid-phase blocking sandwich ELISA:

Six field isolates were compared to the vaccine strain O1/3/93 by one-way liquid-phase blocking sandwich enzyme linked immunosorbent assay (ELISA) using serum collected from bull at 21 days post vaccination according to the method described by Hamblin et al., (1986) and Kitching et al., (1988). Optimal dilution of rabbit, guinea pig hyper immune sera and antiguinea pig conjugated with horseradish peroxidase enzyme was titrated with the vaccine strain O1/3/93. The six serotype O field viruses grown on BHK-21 cell culture also titrated against rabbit/guinea pig hyper immune sera. A fixed concentration of the vaccine strain O1/3/93 and the field viruses, equivalent to a concentration giving an optical absorbance reading of 1.5, was then reacted with 21 days post vaccination bovine serum. The assay measures the residual antigen remaining after overnight reaction between two fold dilutions of the vaccine antiserum and pre-titrated antigen prepared from the six field isolates and the homologous vaccine strain. The serum titer obtained at 50% predetermined virus concentration is used to calculate the relationship "r" values.

Determination of serological relationship "r":

The serological relationship of the isolated virus to a vaccine strain was expressed as an "r" value calculated using the following equation (Pereira, 1978).

$$r = \frac{\text{Serum titer against field virus}}{\text{Serum titer against vaccine strain}}$$

Samuel et al. (1990b and 1993) proposed the following criteria used for interpretation "r" values as follows:

- r = 0 to 0.19. a highly significant serological variation indicating a requirement for a vaccine strain with a closer relationship to the field virus.
- r = 0.2 to 0.39. a significant difference from the reference strain, but protection may be satisfactory if a sufficiently potent vaccine is used.
- r = 0.4 to1.0 not significantly different from the reference strain as measured by the test system used.

RESULTS

Optimal dilution of rabbit, guinea pig hyper immune sera and anti guinea pig conjugated with horseradish peroxidase enzyme titrated against the vaccine strain and the six type O FMD field viruses giving an optical absorbance reading of 1.5 were 1:200,1:200 and 1:2000 respectively. The study was conducted on six type O field isolated from separate outbreaks occurred in three

Governorates. The origins of field isolates are shown in table1. The six viruses were tested for their serological relationship to the vaccine virus O1/3/93. Distribution of the r-values of the field viruses to 21 days post vaccination bovine serum of the currently used vaccine strain is shown in table 1. The six type O isolates revealed r-value ranged between 0.8 and 1 to the vaccine strain O1/3/93. Three virus isolates from Dakahlyea and Sharqiea provinces revealed "r" values of 1 and the fourth had "r" value of 0.8. The two serotype O isolates of Qalubiea province had "ir" values of 0.8 and 1 respectively (Table 1).

Table 1: "r" values of six field isolates of type O foot and mouth disease virus against O1/3/93 21 days post vaccination bovine serum.

Virus number	Governorate	"r" values against O1/3/93 bovine serum
1	Dakahlyea	1
2	Dakahlyea	1
3	Sharqiea	1
4	Sharqiea	0.8
5	Qalubiea	0.8
6	Qalubiea	1
O1/3/93	Vaccine strain	1

DISCUSSION

In countries in which control of FMD relies predominantly on vaccination, the stability of the currently used vaccine in high potency is the only way to protect susceptible animals against FMD outbreaks. High mutation rate of foot and mouth disease (FMD) virus has required the constant monitoring of field strains of FMDV to ensure that vaccine in current used is effective (Kitching et al., 1988). Not only it is essential to have rapid diagnosis of foot and mouth disease field outbreak viruses, but also control measures must be rapidly investigated. The replacement of the classical system of subtyping with a more practical and rapid antigenic assessment has long been overdue. Rapid correlation between field isolates and vaccine strain of FMDV based on a liquid phase blocking sandwich ELISA was reviewed by Kitching et al., (1988, 1989) and Samuel et al.,

(1990b and 1993). Criteria of the antigenic relationship between the vaccine strain and the isolated field virus as ìrî value was established by Pereira (1978) and Rweyemamu (1984). The interpretations of these criteria have been proposed and accepted by Samuel et al., (1990b). Bovine antiserum to the vaccine strain was the most reliable reagent for assessing the ability of the vaccine strain to control disease outbreaks (Kitching et al., 1988 and 1989, Sobrino et al., 1989 and Farag et al., 1998). The protocol described in the study can identify a potential vaccine for controlling an outbreak virus within seven days of the outbreak being typed whereas; the classical methods of subtyping were consuming more than one month (Kitching et al., 1988 & 1989). The present study revealed that all serotype O virus isolates from the three Governorates were more closely related to O1/3/93 vaccine strain with ìrî values ranged from 0.8 to 1. Our results agreed with Kitching et al., 1989, Samuel et al., 1990 a &b and1993 and Farag et al., 1998 who found that a variation of no significance occurred among serotype O1 FMDV field isolates from the Middle East. Accordingly, a good potent vaccine containing strain of O1/3/93 could produce greater protection against the existing field isolates. It can be concluded that although the current used vaccine still the potent vaccine to protect animals against the existing serotype O FMD field isolates, continuing monitoring of the relationship between the vaccine strain and the field viruses will maintain the suitability of the vaccine strain to provide enough protectabililty to the existing field viruses.

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