Vet.Med.J.,Giza. Vol.45, No.4. (1997): 457-465.

STUDIES ON INACTIVATED VACCINE AGAINST INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS (IBRV), I. INFLUENCE OF DEAE-DEXTRAN ON THE YIELD OF IBR VIRUS PROPAGATED ON DIFFERENT CELL CULTURE SYSTEMS

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Received: 14/6/1997
Accepted : 4/10/1997

SUMMARY

Infectivity titre of IBR virus in MDBK cells using monolayer, suspension and roller system revealed that the roller system produced the highest infectivity titre with an increase of log_{10} 1.0 than their corresponding monolayer cell culture. On the other hand, the infectivity titres in cell suspension cultures were log_{10} 0.6-log_{10} 0.8 lower than their corresponding monolayer cell culture system.

Influence of DEAE-dextran for the enhancement of IBR virus infectivity in different cell culture systems, 25 ug DEAE-dextran per ml is considered the safest concentration to theMDBK and VERO cell lines and giving these results:

1- Infectivity titre of IBR virus was markedly increased under the effect of both serial passages and DEAE-dextran in MDBK and VERO cells which showed an increase of log_{10} 1.2 and log_{10} 1.0, respectively more than their respective non treated passages.

2- In th roller system, DEAE-dextran enhanced the growth of virus by about 0.9, 0.7 log_{10} more than growth of untreated virus in both MDBK and VERO cell lines, respectively.

3- The highest titre obtained when using DEAE-dextran MDBK roller system was log_{10} 9.2 $TCID_{50}$/ml.

INTRODUCTION

One of the essential factors in preparing a good and highly immunizing inactivated vaccine is to get a virus of high titre and consequently more antigen in the resulting vaccine as stated by Anderson et al. (1971). The IBR virus is capable of producing a variety of clinical manifestations in

animals. According to type of tissue affected it was a great demand to produce a highly potent inactivated vaccine against it. Greig et al. (1958); Stevens and Goman (1963) and Hahnefeld et al. (1965), described the pathogenicity and characteristic cytopathic effect of IBR virus in MDBK cells and Kolar et al. (1971) propagated IBR virus on MDBK cell line and got the desired concentration of 1 X 10^7 $TCID_{50}$/ml for preparation of inactivated vaccine.

El-Sabbagh (1993) studied some factors influencing the cultivation of IBR virus in cell culture as the sensitivity of different cell culture types.

Polycations such as protamine and DEAE-dextran have been used extensively to facilitate the uptake of infectious viral ribonucleic acid by cells in tissue culture (Pagano and Vaheri, 1965; Smull and Ludwig, 1962). A corresponding enhancing effect on the infectivity of intact virus was noticed for Rift Valley fever in chicken embryo rough (CER) and baby hamster kidney cell (BHK) (Saber et al., 1984).

Similarly, an enhancing effect was noticed with Poliovirus monkey cell system rubella hamster kidney cell system and rabies virus in BHK21 cell culture (Kaplan et al., 1967) as well as respiratroy syncytial virus in Hep-2 cell monolayer (Nomuras, 1968). Plaque enhancement of Newcastle disease virus was recorded by Barohona and Hanson (1968). Such trials encouraged us to use DEAE-dextran for enhancement of IBR virus infectivity in different cell culture systems.

The purpose of the present work is to : 1. study the highest infectivity titer of IBR virus in both MDBK and VERO cells grown in various cultures (monolayer, roller and suspension) to help in choosing the best system. 2. investigate the influence of DEAE-dextran on the yield of IBR virus propagated in different types of cells (MDBK, VERO) grown by different systems (monolayer, suspension and roller).

MATERIAL AND METHODS

1- Virus:

In this study, we used a local Egyptian strain of IBR virus (Abou-Hammed strain) which was previously isolated and identified by Hafez et al. (1976) from calves suffering from respiratory disorders. It was kindly supplied from the Rinderpest Like Diseases Department, Vet. Serum and Vaccine Research Institute, Cairo.

2- Madin Darby Bovine Kidney (MDBK) cell line:

It was obtained from Ames, Iowa Laboratories, USA, Cell monolayers were grown in Eagle's MEM supplemented with 10 percent newly born calf serum (Marcus and Moll, 1968) tested to be free from the non cytopathic (NCP) BVD-MD virus.

3- Vero cell line:

It was obtained from the African Horse Sickness

Department, Vet. Serum and Vaccine Research Institute, Cairo, Egypt. The monolayer culture cells were grown in Eagle's 199 medium supplemented with 10 percent newly born calf serum.

4- Media and Chemical reagents:

a- Minimum essential medium Eagle's (MEM):

Modified with Eagle's salt without sodium bicarbonate was obtained from Flow Laboratories, UK. It was used for growing MDBK line culture.

b- Growth 199 medium:

Difco tissue culture medium 199 (Morgan et al., 1950). It could be used as a growth medium for the propagation of VERO cells as well as a maintenance medium.

5- Cell culture:

a- Monolayer cell culture:

MDBK and VERO cells were grown in monolayers in Roux bottles. When intended for vaccine production for virus titration the cells were grown in test tubes.

b- Roller culture:

MDBK and VERO cell cultures were propagated in 800 ml roller bottles (Struma blood plasma bottles, Arthur Thomas Co. Philadelphia, Pa) rotated at 8 rpm. Minimum essential medium Eagle's and medium 199 were used for growing MDBK and VERO cell lines respectivley, and after 48 hours, the total cell concentration in the average roller bottle was about 8 X 10^7 cells with 100 ml growth medium supplemented with 10% calf serum, antibiotic and tryptose phosphate.

c- Cell suspension:

MDBK and VERO cell cultures were grown as spinner cultures is 500 ml volumes in bottles containing teflon coated magnetic bar and placed over a magnetic stirrer. Growth medium supplemented with 10% calf serum, antibiotics, tryptose phosphate were used. Viable cells as determined by trypan blue stain were counted in hemocytometer chambers.

d- Cell counting and viability:

The cells were counted by using modified Fuchs-Rosenthal chamber according to the following equation.

Cell number per one square X dilution rate X depth of the slide X 10^3: cell number counted X 10^6 X ml. The cell viability was determined by using 0.05% trypan blue. The dead cells were stained blue, while living ones remained unstained.

6- Diethyl amino ethyl (DEAE) dextran:

It was obtained as powder from Pharmacia, Uppsaler, Sweden. It was prepared in a solution of 10µg/ml by dissolving 0.1 gm of DEAE-dextran in 100 ml of double distilled water. This was autoclaved and used at a final concentration of 25 µg/ml for both types of cells used in this study.

Formula:

0.1 gm DEAE-dextran in 100 ml double distilled water (DDW).
1/10 x 1000 mg D-dextran in 100 ml DDW.
100 mg D-dextran in 100 ml DDW.
100 x 1000 ug D-dextran in 100 ml DDW.
1000 x 100/100 = 1000 ug/ml.
0.1 ml of DDW contains 100 ug D-dextran.
0.25 ml of D-dextran + 0.75 ml of virus suspension.

7- Titration of IBR virus:

MDBK and VERO cells were grown in monolayer, suspension and roller systems as previously described in methods. The total calculated cell numbers were 5 x 10^7, 8 x 10^7 in both monolayer and roller systems respectively and about 2 x 10^6 per ml in suspension system. The cultures were infected with a fixed dose of virus. After 2 hours adsorption, maintenance medium was added to all cell culture systems and incubated at 37°C for about 72 hours. Then the virus harvested was assayed for infectivity titres in the respective cell culture. The titre was expressed as $\log_{10}$ $TCID_{50}$.ml virus using the formula of Reed and Muench (1938).

EXPERIMENT and RESULTS

1- Infectivity titres of IBR virus in MDBK and VERO cells using monolayer, suspension and roller systems:

Results of this study are illustrated in Table (1) which demonstrated that the roller system produced the highest infectivity titre for both cell types. There is an increase of $\log_{10}$ 1.0 and 1.2 for MDBK and VERO cell types, respectively than their stationary monolayer cell culture. On the other hand, the infectivity titres of IBR virus in cell suspension culture were $\log_{10}$ 0.6 $\log_{10}$ 0.8 lower than their corresponding monolayer cell culture system.

Table (1) : Influence of cell culture system on the final infectivity titre of IBR virus grown in MDBK, VERO cell line.

Type of cell used	Titre expressed in $\log_{10}$ $TCID_{50}$ / ml		
	Monolayer system	Suspension system	Roller system
MDBK	$10^{7.4}$	$10^{6.6}$	$10^{8.4}$
VERO	$10^{5.6}$	$10^{5.0}$	$10^{6.8}$

. Cytotoxic effect of different concentrations (DEAE-dextran on both type of cell cultures:

ifferent concentrations of DEAE-dextran g/ml) were used with both types of cells chosen r virus propagation in order to determine the ptimum quantity of the chemical needed to hance the virus propagation without inducing xic effect to the cells used. Results are given in able (2).

From the obtained data, it can be seen that 100% cytotoxic effect was noticed on treatment of MDBK and VERO cell cultures with 200 and 100 ug/ml while, 50% and 25% cytotoxic effect appeared by other doses, respectively. A dose of 25µg/ml EEAE-dextran produced no cytotoxic effect.

3- Effect of DEAE-dextran on the growth of IBR virus in 2 types of cells:

1.5 ml of virus and 0.5 ml of DEAE-dextran (100 ug/ml) were mixed together to get a final concentration 25 ug/ml of DEAE-dextran. For both types of cell culture, two prescription bottles were inoculated with the mixture, two prescription bottes of both types of cell; each was inoculated with 0.2ml of virus alone. The virus harvested was assayed for virus infectivity 72 hours post infection. Seven successive passages in the presence or absence of DEAE-dextran were carried out and the results are given in Table (3). It is clear that the effect of 7 serial passages of IBR virus in both cell cultures revealed an average of 1.5 and 2.3 $\log_{10}$ in MDBK and VERO cell cultures, respectively in fourth passage in the absence of DEAE-dextran. And when

Table (2) Effect of various concentrations of DEAE-dextran on both type of cell cultures.

DEAE-dextran in ug/ml	Types of cells		Percentage of cytotoxic effect
	MDBK	VERO	
200	++++	++++	100 %
100	++++	++++	100 %
75	++±	++	50-75 %
50	++	+	25-50 %
30	+	-	25 %
25	-	-	No toxicity
20	-	-	No toxicity
10	-	-	No toxicity

++++ : 100 % cytotoxic effect.
+++ : 75 % cytotoxic effect.
++ : 50 % cytotoxic effect.
+ : 25 % cytotoxic effect.
- : No toxicity.

Table (3) : Comparative titration of IBR virus passaged successively in both types of cells in presence and absence of DEAE-dextran.

No. of passage	Titre expressed in log_{10} $TCID_{50}$/ml in both cell type			
	MDBK		VERO	
	+ D	- ve	+ D	- ve
1	6.5	5.5	3.5	3.0
2	7.0	5.8	4.5	3.5
3	7.8	6.5	6.2	4.5
4	8.2	7.0	6.2	5.3
5	8.3	7.4	6.5	5.5
6	8.5	7.4	6.5	5.6
7	8.3	7.3	6.5	5.5

DEAE-dextran was added to both cells in the first passage, titres increased about log_{10} 1.3 and 1.7 at third passage and log_{10} 1.2 and 0.9 at 4th passage for MDBK and VERO cells, respectively than the untreated with DEAE-dextran and the titre was stable after the 4 th passage since further passages provoked little or no increase in the virus titre.

4- The effect of DEAE-dextran on the final titre of IBR virus propagated in different cell culture systems:

In this experiment, both MDBK and VERO cell types were propagated under the following systems: monolayer suspension and the roller system using the previous recommended conditions with and without DEAE-dextran.

Results of this investigation are illustrated in Table (4), where it was found that MDBK gave higher titres than VERO cell whether propagated as monolayer or suspension or by means of the roller system. However, there was always difference of about more than 1 log_{10} when DEAE-dextran was added to the VERO cells.

The results indicated that the roller system is much better than the other two systems whether DEAE-dextran was added or not, since the roller system showed an increase of about 0.9 and 0.7 log_{10} than the monolayer system and about 2.5 and 2.2 log_{10} than the suspension system of MDBK and VERO cell lines, respectively.

Table (4) : Effect of cell culture system on the final virus titre in the presence and absence of DEAE-dextran.

Type of cell culture used	Titre expressed in log_{10} $TCID_{50}$ / ml					
	Monolayer system		Suspension system		Roller system	
	+ D	- D	+ D	- D	+ D	- D
MDBK	8.3	7.4	ND	6.6	9.2	8.4
VERO	6.5	5.6	ND	5.0	7.2	6.8

+ D : Presence of DEAE-dextran.
- D : No DEAE-dextran was added.
ND : Not Done.

DISCUSSION

The kinetics of virus cell interaction is a complex, and implying considerable difficulties when we study the effect of several factors on final virus yield to choose optimal conditions. This includes the ratio of the inoculated particles to the cells, the composition of the maintenance medium e.g. presence or absence of DEAE-dextran, the conditions of virus yield, and several others.

Concerning the influence of cell culture system on the final infectivity titre of IBR virus grown in MDBK and VERO cell lines. The results in table (1) indicated that the roller system produced the highest infectivity titre with an increase of 1.0 and 1.2 log_{10} for MDBK and VERO cells than monolayer system. Yet, the suspension system gave an infectivity titre of 0.6 to 0.8 log_{10} lower than the monolayer system.

The lower infectivity titres in suspended cell culture may be attributed to the action of two factors. The first was the decrease in the viability of the suspended cells and the second factor is the rapid release of the virus from the cell and virus stability may be affected by other culture medium and environment as confirmed by Ferrari et al. (1981) and Stevens et al. (1963).

The rapid loss of viability obtained with suspended culture does not correlate well with the development of cytopathological changes of the virus in infected monolayer on or it may be a

result of temperature of incubation or by the action of culture medium as pH or presence of horse serum.

DEAE-dextran was used in this study with the aim of getting higher titres of IBR virus grown in both types of cells (MDBK and VERO cells) propagated by different techniques. In a preliminary step, the non cytotoxic dose of DEAE-dextran for each type of cell was evaluated (Table 2). A dose of 25 ug/ml was selected for virus enhancement according to Saber et al. (1984).

According to the results in table (3) IBR virus was propagated in MDBK and VERO cells for seven successive passages without using DEAE-dextran, there was a progressive increase to reach a titre of 7.4 and 5.6 $\log_{10}$, respectively by the 6th passage. When DEAE-dextran was added, the respective titres were 8.3 and 6.5 $\log_{10}$ after 5 passages, then remained constant afterwards.

Results of the effect of DEAE-dextran on the final titre of IBR propagated in different cell culture systems revealed once more that DEAE-dextran caused an increase in the final virus titre with the used system (monolayer or roller) yet, the increase was about 0.8 $\log_{10}$ for both monolayer and roller systems. This phenomenon was explained by Kaplan et al. (1967) as that the polycations could possibly act by binding to the cellular surface thereby creating a favourable ionic charge for virus attachment. Another explanation is by completing with the virus particles (thereby allowing them to attach to the cell surface more efficiently) (Pagona and Vaheri, 1965). This result is very important in preparing inactivated vaccine according to Anderson et al. (1971), who indicated that higher antigen doses maintained serum antibody for a longer period and this was associated with the change from IgM to IgG production. Many authors explained the role of DEAE-dextran for improvement of the immune response of vaccines and suggested that the adjuvant activity of DEAE-dextran could be due to a membrane effect on the immuno-competent cells to become antibody producer (Wittmann, 1970; Anderson et al. 1971).

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