EFFECT OF DEXTRAN ON THE INFECTIVITY TITER OF BOVINE EPHEMERAL FEVER VIRUS PRODUCED ON DIFFERENT CELL CULTURES

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SUMMARY

The present study was a trial to increase the infectivity titer of BEF virus in two of the recommended cell lines for virus propagation (VERO and BHK_{21}) with the acceleration of onset of CPE and harvestation time, using DEAE-dextran in order to obtain a maximum titer of BEF virus in cell cultures and consequently allowing massive production of the vaccine.

The local isolate of BEF virus used for vaccine production was passaged through VERO and BHK_{21} cell cultures in two methods. In the first one, the virus was inoculated into all cultures without the tested material while in the second method, DEAE-dextran was added to the virus inoculums at a concentration of 25, 50, 75 and 100 mg/ml. Virus inoculation was carried out on confluent cell sheets allowing virus adsorption for one hour at 37°c. It was noticed that the onset of CPE was earlier in case of the use of treated inoculums than in the case of the use of untreated inoculums in both VERO and BHK_{21}cells. Also the virus titers were higher with the use of DEAE-dextran with the concentrations of 50-75 mg/ml than in case of its absence. So, the use of DEAE-dextran with the best concentration could be recommended to increase the virus titer and accordingly increase the vaccine production and decrease its cost.

INTRODUCTION

Bovine ephemeral fever virus was considered as unclassified Rhabdovirus with known four serotype: DDP63, CSIR0368, DDP61 and EUK-11 *(Gard, et al. 1984 and Kaneko, et al.1986)*. Recently the virus was classified as the type species of the genus Ephemerovirus with Adelaide river virus, Berrinah virus, Kimberley, Malakal and Puchong viruses *(Wunner, et al. 1995)*.

Usually, adaptation of viruses to different cell cultures represents an interesting field of virology research. Many workers tried successfully to adapt BEFV to cell cultures like *Inaba, et al. (1968)* who adapted their strain YH hamster adapted virus to BHK_{21}-clone21 cells and designated it as YHK

strain. *Snowdon (1968 and 1970)* inoculated BEFV from mouse brain or bovine leucocyte suspension in BHK_{21} cells and *Westhuizer (1967)* adapted BEFV-1strain to BHK_{21}-clone 13 while *Heuschele (1970)* replicated the virus in Vero and MS monkey kidney cells. *Kaplan, et al. (1967)* reported that the adsorption and penetration of the virus can be enhanced by the presence of polions, diethylaminoethyl-dextran (DEAE-dextran), and protamine sulfate.
DEAE-dextran enhances the uptake of nucleic acids into cells by interacting with both nucleic acid and the cell surface. The transport of DNA into cultured cells can be increased three to ten times by the use of this product *(Borenfreund, 1973 and Pagano, 1970).*
The enhancement of viral infectivity in cell culture systems by DEAE-dextran is well documented for a number of viruses *(Pagano and Mccutchan,1969; Rossi and Kiesel , 1978 and Sasaki, et al. 1981).*

In order to obtain a maximum titer of BEF virus in cell cultures and consequently allowing massive production of the vaccine, the present study was planned to increase the susceptibility of cell cultures used for such purpose (BHK_{21} cells and VERO cells) and increase the infectivity titer using DEAE-dextran.

MATERIALS AND METHODS

1-Virus:
The virus used in the present study was the local strain of bovine ephemeral fever Abbassia virus strain "BEF-AVS" *(Soad, et al. 2001).*

2-Cell cultures:
Two different cell lines were used in the present study and were supplied by the department of Pet animal vaccine research, Vet. Ser. Vac. Res. Inst., Abbassia, Cairo. These cell lines are:
a-baby hamster kidney BHK_{21}-clone 13 cell culture *(Macpherson and stocker, 1962).* b- Vervet Monkey kidney (Vero) cells *(Yasumara and Kawatika, 1963)*

3-DEAE-dextran solution:
A solution of diethylaminoethyl-dextran of molecular weight 500,000 was prepared by dissolving 100 mg in 1ml of 0.25M Tris-Hcl buffer of PH 8.2. This solution was sterilized by autoclaving and its pH was adjusted to 7.6-7.8, according to *Anderson, et al. (1971).*

4-Virus propagation:
BEV-AVS2000 was propagated in each cell line according *to the manual of Laboratory diagnosis of viral diseases (1977)* without adding of DEAE-dextran.Other cell culture were infected with BEF virus using DEAE-dextran at different concenteration(25,50,75 and100 mg/ml).

5-Virus titration:
It was carried out as described by *Rossiter and Jessett (1982)* using a microtitre technique, while the virus titer was calculated according to *Reed and Muench (1938).*

RESULTS AND DISCUSSION

Many viruses demonstrate their presence in susceptible cells by producing different degenerative changes in the infected cells. These cytopathic effects (CPE) are usually characteristic for each virus and include morphological changes of the cells end mainly with complete cellular degeneration *(Manual of laboratory diagnosis of viral diseases, 1977).*

Usually viral vaccine producers hope to increase their production with a maximum possibility of cost reduction. One of the methods that help in such purpose is the trial to increase virus infectivity titers. So the main goal of the present study was a trial to increase the infectivity titer of BEF virus in two of the recommended cell lines for virus propagation (VERO&BHK_{21}) with the acceleration of onset of CPE and harvestation time, using DEAE-dextran.

The local isolate of BEF virus used for vaccine production was passaged through VERO and BHK_{21} cell cultures in two methods. In the first one, the virus was inoculated into all cultures without the tested material while in the second manner, DEAE-dextran was added to the virus inoculums at a concentration of 25, 50, 75 and 100 mg/ml. Virus inoculation was carried out on confluent cell sheets allowing virus adsorption four one hour at 37°c then the infected cells were washed twice with PBS and supplemented with maintenance medium. It was noticed that the onset of CPE appeared earlier in case of the use of treated inoculums than in the case of the use of untreated inoculums in both VERO & BHK_{21}cells. Also the virus titers were higher with the use of DEAE-dextran than in case of its absence (table1&2).

These findings could be explained on the basis of the fact that virus adsorption and penetration can be enhanced by the presence of diethyl aminoethyl-dextran *(Kaplan, et al. 1967).* This fact allows accordingly the production of viral infected fluid with high titer.

Regarding the use of different concentrations of DEAE-dextran, it was noticed that the best concentration is 50mg/ml while the lower concentration yielded lower virus titer and higher concentrations revealed no significant changes in the viral titer. Similar results were obtained by *Kaplan, et al. (1967)* who found that DEAE-dextran (50mg/ml) improved the susceptibility of cell culture to rabies virus (which is a member of the Rhabidoviridae with BEF virus as mentioned by *(Murphy, et al.1999).*

Also, *Borenpreund (1973);Pagano(1970)* stated that DEAE-dextran enhances the uptake of nucleic acids into cells by interacting with both nucleic acid and cell surface.

Pagano and Mc Cutchan (1969); Rossi and Kiesel (1978) and Sasaki, et al. (1981) showed that the viral infectivity of a number of viruses in cell culture systems was enhanced by DEAE-dextran. *Fox, et al. (1977)* found that

in the presence of DEAE-dextran with the nucleic acids, nucleotides may form complexes with DEAE-dextran on the basis of their change differences, thereby facilitating their entry into the cells.

All these data agree with and confirm the obtained results in the present work and it could be suggested that the use of DEAE-dextran will help in the increasing of BEF vaccine prodution.

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Table (1): Effect of DEAE-dextran on BEF virus infectivity titer in BHK_{21} cell culture

Used concenteration	BEF virus titer /log_{10} $TCID_{50}$/ML		Time of harvestation
	Control of BHK cells	Subjected cells to the test	
25mg/ml	10^6TCID_{50}/ml	6.5	72 hours
50mg/ml		7.5	48 hours
75mg/ml		7.6	48 hours
100mg/ml		7.5	48 hours

Table (2): Effect of DEAE-dextran on BEF virus infectivity titer in VERO cell culture

Used concenteration	BEF virus titer /log_{10} $TCID_{50}$/ML		Time of harvestation
	Control of VERO cells	Subjected cells to the test	
25mg/ml	$10^{5.5}TCID_{50}$/ml	6.0	120 hours
50mg/ml		7.0	96 hours
75mg/ml		7.1	108 hours
100mg/ml		7.0	96 hours

الملخص العربى

تأثير مادة الديكستران على القوة العيارية لفيروس حمى الثلاثة أيام المنتج فى مزارع نسيجية مختلفة

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تعتبر هذه الدراسة محاولة لزيادة القوة العيارية لفيروس حمى الثلاثة أيام فى نوعين من المزارع النسيجية (خلايا كلى القرد الأخضر الأفريقى وخلايا كلى رضيع اليربوع السورى) بإسراع وقت ظهور التأثير المرضى للفيروس فى الخلايا ووقت جمع السائل الفيروسى باستخدام مادة الديكستران للحصول على أعلى معيار ممكن للفيروس للمساعدة فى زيادة إنتاج اللقاح

وقد تم حقن العترة المحلية من الفيروس فى الخلايا النسيجية المذكورة مرة بدون الديكستران وأخرى معه بتركيزات 100،75،50،25 مليجرام/مل من فيروس الحقن حيث تبين أن وجود الديكستران يساعد على ظهور تأثير الفيروس فى الخلايا المحقونة أسرع منه فى حالة عدم وجود هذه المادة مع ملاحظة أن معيار الفيروس يكون أعلى فى الحالة الأولى منه فى الحالة الثانية كما يمكن القول بأنه لا توجد فروق محسوسة فى معيار الفيروس الناتج مع تركيزات 50، 75،100 مليجرام/مل بينما أعطى التركيز 25مليجرام/مل تأثيرا أقل فى نوعى الخلايا المستخدمين الأمر الذى يحبز معه استخدام افضل التركيزات (50-75 مليجرام/مل)