Trials for increasing the infectivity titer of fowl pox vaccines prepared on SPF embroynated chicken egg and tissue culture

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Abstract:

The present work was designed to study the effect of DEAE-dextran and Magnesium Chloride (MgCl) on fowl pox virus vaccine (FPV) in order to obtain a maximum titer allowing the massive production of the vaccine.

Fowl pox virus; used for vaccine production; was propagated on embroynated chicken eggs (ECE) and chicken embryo fibroblast (CEF), the virus was inoculated in ECE and CEF without DEAE-dextran nor MgCl, and with DEAE-Dextran and MgCl separately. The time of harvestion was early as one day post inoculation in CEF. The virus titer were higher in case of treated inoculums with DEAE-dextran and MgCl reaching (6.2 and 7.5 $log_{10}TCID_{50}$/ml respectively) on CEF and (6.2 and 6.7 $Log_{10}EID_{50}$ respectively) on ECE. Fowl pox vaccines (FPV) were prepared before and after DEAE-Dextran and MgCl treatment. The application of quality control assays revealed the safety, sterility and potency of the prepared vaccines. Immune response of the prepared FPV vaccines were evaluated in chickens using virus neutrization test (VNT). It was found that, the use of DEAE-Dextran and MgCl with the recommended concentrations could result in increasing the virus titer with reduction in the time of harvestation and accordingly increase the vaccine production and decrease its cost.

Introduction

Fowl pox virus (FPV) is a member of genus Avipox which is brick shaped and measured 270 x 180 nm ***(Tripathy and Reed, 2008).***

Fowl pox is characterized by the formation of proliferative lesions and scabs on the skin, and diphtheritic lesions in the upper parts of the digestive and respiratory tracts which is fetal form. Modified live FPV vaccines of chicken embryo or avian cell culture origin are recommended for protection of fowl from FPV infection in endemic areas ***(OIE, 2012).***

Plaque formation by certain rhinoviruses in HeLa cells was enhanced by higher concentrations

of magnesium in the overlay medium *(Fiala and Kenny, 1966; Stott and Tyrrell, 1968)*. Susceptibility of monkey kidney cells to poliovirus and the release of rhinovirus from HeLa cells was also greatly enhanced in the presence of high levels of MgCl *(Fiala & Kenny, 1967)*. MgCl enhances plaque formation by human adenoviruses in HeLa cell monolayer and that effect is due to an increase in the rate of virus release *(Russell et al., 1970).* Magnesium ions enhance plaque formation (replication of progeny virus) of lentogenic strains of Newcastle disease virus (NDV) *(Sahle et al, 2002).*

The enhancement of viral infectivity in cell culture systems by DEAE-Dextran is well documented for a number of viruses *(Pango and Mccutchan, 1969 and Sasaki et al, 1981)*. DEAE-dextran increases the DNA transfication in primary cultured adherent human macrophage *(Carel et al., 1997)*. The onset of CPE appeared earlier and virus titers were higher in case of the use of treated inoculums with DEAE-Dextran in Bovine Ephemeral Fever (BEF), camel pox virus (CPV) and avian influenza virus (AI) *(Ayatollah et al, 2007, Zeneib Salama. 2006 and Eman et al, 2011).* Also DEAE-Dextran improves in-vitro cultivation of swine influenza virus *(Margot et al., 2005)* and enhanced retro virus infection efficiency about 3 folds *(Lee et al., 1996).* The efficacy of introduction for exogenous gene into tissue culture was improved using DEAE-dextran *(Yuki et al, 2009).*

The present study was planned to evaluate the effect of MgCl and DEAE-Dextran on FPV used for vaccine production, in a trail to obtain a maximum titer of virus yield consequently allowing massive production of the vaccine.

Materials and methods

1. Virulent and vaccinal fowl pox viruses

Vaccinal (Baudate, egg adapted strain - SATRO Italy company batch no 303092, FPV- CEF adapted strain) and Virulent FPV were supplied from Pox Research Department VSVRI and CLEVB for vaccination and challenge the chickens according to *(OIE, 2012).*

2. DEAE-Dextran and MgCl solutions

Different concentrations of DEAE-Dextran (obtained from ICN Biomdical ICN) and MgCl (obtained from Sigma chemical company) 10ug/ml, 20ug/ml, 25ug/ml, 30ug/ml, 40ug/ml, 50ug/ml, 75ug/ml, 100ug/ml, 150ug/ml and 200ug/ml were prepared and sterilized by autoclaving according to ***Anderson et al (1971).***

3.Tissue culture

Primary CEF was kindly supplied by CLEVB for propagation and titration of FPV with and without DEAE-Dextran and MgCl according to ***Charles and Cunnimgham (1973).***

4. Specific pathogenic free embroynated chicken eggs

SPF embroynated chicken eggs (ECE) kindly supplied from Pox Research department VSVRI and used for propagation and titration of FPV with and without DEAE-Dextran and for detecting of FPV neutralizing antibodies in egg according to ***Namaa Abdel-Aziz. (1998).***

5. Experimental chickens

Two hundred and seventy five Specific pathogen free (SPF) chickens 2 weeks old supplied by CLEVB, were divided into 11 groups 25 chicken for each.

1- Group no (1) vaccinated with field dose ($10^3 log_{10} EID_{50}$/ml) of treated FPV with MgCl prepared on ECE.

2- Group no (2) vaccinated with field dose ($10^3 log_{10} TCID_{50}$/ml) of treated FPV with MgCl prepared on CEF.

3- Group no (3) vaccinated with field dose ($10^3 log_{10} EID_{50}$/ml) of treated FPV with DEAE-Dextran prepared on ECE.

4- Group no (4) vaccinated with field dose of ($10^3 log_{10} TCID_{50}$/ml) treated FPV with DEAE-Dextran prepared on CEF.

5- Group no (5) vaccinated with 10x field dose ($10^4 log_{10} EID_{50}$/ml) of treated FPV with MgCl prepared on ECE.

6-Group no (6) vaccinated with 10x field dose ($10^4 log_{10} TCID_{50}$/ml) of treated FPV with MgCl prepared on CEF.

7- Group no (7) vaccinated with 10x field dose ($10^4 log_{10} EID_{50}$/ml) of enhanced FPV with DEAE-Dextran prepared on ECE.

8- Group no (8) vaccinated with 10x field dose ($10^3 log_{10} TCID_{50}$/ml) of enhanced FPV with DEAE-Dextran prepared on CEF.

9- Group no (9) vaccinated with field dose($10^3 log_{10} EID_{50}$/ml) of non enhanced FPV prepared on ECE.

10- Group no (10) vaccinated with field dose ($10^3 log_{10} TCID_{50}$/ml) of non enhanced FPV prepared on CEF.

11- Group no (11) control non vaccinated chickens.

NB: Groups (1-2-3-4-11) were challenged with virulent FPV with titer 10^3 log_{10} EID_{50} /ml 2 weeks post vaccination .

6 -Serum samples

Blood samples were collected before and after vaccination from wing vein of chickens and left for coagulation and serum collection to measure the protective level of Fowl Pox antibodies by VNT.

7-Vaccination and challenge

Vaccination and challenge were carried out according to ***OIE (2012)***.

8- Cytotoxicity test for DEAE-dextran and MgCl on CEF and ECE

The toxic effect of various concentrations of DEAE-dextran and MgCl (200-150-100-75-50-40-30-25-20-10 ug/ml) were tested in ECE and CEF according to ***Ayatollah et al (2007)***

9-Studying the effect of various concentration of DEAE-dextran and MgCl on the titer of FPV on CEF cell line

Fowl pox virus was propagated and titrated in CEF cell line without and with addition of DEAE-Dextran and MgCl in different concentrations according to ***Ayatollah et al (2007).*** Titrating of virus infectivity were carried out according to the method described by ***(Reed and Munch, 1938)***

10-Quality control of treated and non treated FPV vaccines

Sterility, safety and potency testes were applied on treated and non treated FPV vaccines as mentioned in ***OIE (2012)***.

11-Virus neutrization test

Virus neutralization test (VNT) was applied on serum samples collected from vaccinated chickens with treated FPV vaccines and currently used FPV vaccine according to ***OIE (2012).***

Results

1- Cytotoxic effect of DEAE-dextran and MgCl on CEF and ECE

Table (1) shows that DEAE-Dextran concentrations higher than 75ug/ml were toxic for CEF and concentrations above 20ug/ml were toxic for ECE, while MgCl concentrations above 40 ug/ml were toxic for CEF and concentrations above 25 ug/ml were toxic for ECE.

2-Effect of different concentrations of DEAE-dextran and MgCl on the infectivity titer of FPV on CEF cell.

The best concentration of DEAE-dextran were 75ug/ml and 50ug/ml, while the lower concentrations yield lower virus titer. Higher concentrations more than 50ug/ml revealed no significant changes in viral titer in CEF, while the best concentrations of MgCl were 30ug/ml and 40 ug/ml as lower concentrations yields lower virus titer. Higher concentrations more than 40ug/ml revealed no significant virus titer increase.

3- Effect of DEAE-dextran and MgCl on different passages of FPV propagated on CEF and ECE.

FPV mixed with DEAE Dextran and inoculated in CEF and ECE for 10 passages at concentration of 50ug/ml for CEF and 25ug/ml for ECE. The results are presented in table (3) showed that the virus titer increased gradually from 5.7 log $_{10}$ $TCID_{50}$ /ml in first passage till reach 6.7 $\log_{10}$ $TCID_{50}$ /ml in the 9th passage however in ECE, the virus titer increased gradually from 5.2 $\log_{10}$ EID_{50} /ml in first passage till reach 6.2 $\log_{10}$ EID_{50} /ml in the 8^{th}passage

Regarding the effect of MgCl on FPV inoculated in ECE and CEF. Data recorded in table (3) revealed that, the virus titer on CEF increased gradually from 6.0 $\log_{10}$ $TCID_{50}$ /ml in first passage to 7.5 $\log_{10}$ $TCID_{50}$ /ml at 8th passage. While in ECE using of 20 ug/ml of MgCl increased the virus titer from

5.2 $\log_{10}$ EID_{50} /ml in the first passages till reach 6.7 $\log_{10}$ EID_{50} /ml in the passage number 8.

4-Time of harvestaion for FPV propagated on CEF with DEAE-Dextran and MgCL

To determine the harvesting time of FPV in which the virus titer was maximum after treatment with DEAE-Dextran and Mgcl CPE was used as indicator. Results presented in table (4) indicated that, the harvestion time for treated FPV propagated on CEF decreased gradually with increasing the number of passages when the virus treated with either DEAE-Dextran or Mgl. The best time for harvesting of FPV was 4 days PI when treated with 50ug/ml DEAE-Dextran at the 6th passage, however, the time for collection of FPV when treated with 30ug/ml MgCl was 4 days PI at the passage no 3.

5-Quality control of the prepared enhanced FPV vaccines

Sterility test: Bacterial culture of treated FPV vaccines with DEAE-dextran and MgCl on both ECE and CEF proved to be free from any bacterial and fungal contamination.

Safety test: Inoculation of treated FPV vaccine in SPF chickens with 10 times of the recommended dose proved that the produced vaccine was safe to be used in chickens. where the vaccinated birds did not show any undesirable symptoms refer to FP.

Challenge test: Vaccination of SPF chickens with treated FPV vaccines by using wing web stabbing method and challenged with virulent FPV revealed that, the protection percent of vaccinated chickens with treated FPV vaccines with MgCl and DEAE-dextran propagated on ECE and CEF were 96%, 92%, 96% and 92% respectively, as shown in table (5).

5-Potency test of the treated vaccines in chickens (Virus neutrization test –Alpha procedure)

The results were presented in table (6), it was noticed that, chicken vaccinated with field dose (3.7 $\log_{10}TCID_{50}$/ml) of non treated FPV propagated on ECE and CEF gave similar NI like the field doses of treated FPV on ECE and CEF with DEAE-dextran and MgCl. Moreover the NI of the vaccinated chickens with treated FPV vaccines (10x field dose 4.7 $\log_{10}$ $TCID_{50}$/dose) revealed slight high NI.

Table (1): *Effect of various concentrations of DEAE-Dextran and MgCl on CEF and ECE*

Used conc	DEAE-Dextran cytotoxity		MgCl cytotoxity	
	CEF	ECE	CEF	ECE
200ug/ml	100%*	100%*	100%*	100%*
150ug/ml	75%	100%	100%	100%
100ug/ml	50%	100%	100%	100%
75ug/ml	0%	100%	50%	100%
50ug/ml	0%	100%	25%	75%
40ug/ml	0%	75%	0%	50%
30ug/ml	0%	50%	0%	25%
25ug/ml	0%	25%	0%	0%
20ug/ml	0%	0%	0%	0%
10ug/ml	0%	0%	0%	0%

*Cytoxocity percent was measured according to the percent of the damage and destruction in the CEF cell sheet and embryo death in ECE.

Table (2): *Effect of various concentrations of DEAE-dextran and Mgcl on the infectivity titer of FPV on CEF cell*

DEAE-Dextran Used conc	Titer of FPV in CEF with DEAE-dextean expressed as log $_{10}$TCID$_{50}$/ml	MgCl used conc	Titer of FPV in CEF with Mgcl expressed as log $_{10}$TCID$_{50}$/ml
0 ug	5.5	0 ug	5.5
10 ug	5.5	10 ug	5.5
25 ug	5.5	20 ug	5.7
50 ug	5.7	30 ug	6.0
75 ug	5.7	40 ug	6.0

Table (3): *Titration of serial FPV passage in CEF and ECE presence and absence of MgCl and DEAE-dextran*

No of passages	Titer expressed as $\log_{10}$ TCID $_{50}$/ml in CEF			Titer expressed as $\log_{10}$ EID $_{50}$/ml in ECE		
	Treated FPV in CEF with DEAE-dextran 50ug/ml	Treated FPV in CEF with MgCl 30 ug/ml	Non-Treated FPV in CEF	Treated FPV in ECE with DEAE-dextran 25ug/ml	Treated FPV in ECE with MgCl 20ug/ml	Non-Treated FPV in ECE
1	5.7	6.0	5.5	5.2	5.2	5.0
2	5.7	6.2	5.5	5.2	5.5	5.0
3	6.0	6.5	5.7	5.2	5.5	5.2
4	6.2	6.7	5.7	5.5	5.7	5.2
5	6.2	6.7	5.7	5.7	6.0	5.2
6	6.5	7.0	6.0	5.7	6.2	5.5
7	6.5	7.2	6.0	6.0	6.5	5.5
8	6.5	7.5	6.0	6.2	6.5	5.5
9	6.7	7.5	6.2	6.2	6.7	5.7
10	6.7	7.5	6.2	6.2	6.7	5.7

Table (4): *Time of harvestaion for enhanced and non enhanced FPV in CEF.*

	Treated FPV with 50 ug/ml DEAE-Dextran					Treated FPV with 30ug/ml Mgcl					Non treated FPV				
No of passage	1st day	2nd day	3rd day	4th day	5th day	1st day	2nd day	3rd day	4th day	5th day	1st day	2nd day	3rd day	4th day	5th day
1	-	+	++	++	+++	_	+	++	++	+++	_	+	++	++	+++
2	-	+	++	++	+++	_	+	++	++	+++	_	+	++	++	+++
3	-	+	++	++	+++	+	++	++	+++		_	+	++	++	+++
4	-	+	++	++	+++	+	++	++	+++		_	+	++	++	+++
5	-	+	++	++	+++	+	++	++	+++		_	+	++	++	+++
6	+	+	++	+++		+	++	++	+++		_	+	++	++	+++
7	+	+	++	+++		+	++	++	+++		_	+	++	++	+++
8	+	++	++	+++		+	++	++	+++		_	+	++	++	+++
9	+	++	++	+++		+	++	++	+++		_	+	++	++	+++
10	+	++	++	+++		+	++	++	+++		_	+	++	++	+++

-= no CPE

+ = rounding of cells

++ =50% sheet destruction (degenerative change and necrosis)

+++ = 75% sheet destruction (necrosis)

Table (5): *protection percent in vaccinated and control chicks after challenged with virulent FPV virus*

Time post challenge	Chickens groups	No. of challenged Chickens/group	No. of birds showing lesion post challenge	Protection percent (%)
3 weeks post challenge	1	25	1	96%
	2	25	2	92%
	3	25	1	96%
	4	25	2	92%
	5	20	20	0%

Group (1) vaccinated SPF chickens with Treated MgCl FPV ECE.
Group (2) vaccinated SPF chickens with Treated MgCl FPV on CEF.
Group (3) vaccinated SPF chickens with Treated DEAE-Dextran FPV ECE.
Group (4) vaccinated SPF chickens with Treated DEAE-Dextran FPV on CEF.
Group (5) non vaccinated SPF chickens

Table (6): *Results of the VNT for chickens vaccinated with treated, non treated FPV and non vaccinated chickens*

	NI of chickens vaccinated with treated FPV field dose (3.7 log $_{10}$ TCID$_{50}$ /ml /dose)				NI of chickens vaccinated with treated FPV field dose (4.7 log$_{10}$ TCID$_{50}$ /dose) 10x field dose				NI of chickens vaccinated with non treated FPV field dose (3.7log$_{10}$ TCID50 /dose)		NI of control chickens
WPV	FPV in ECE with MgCl	FPV on CEF with MgCl	FPV on ECE with DEAE-dextran	FPV on CEF with DEAE-dextran	FPV in ECE with MgCl	FPV in CEF with MgCl	FPV in ECE with DEAE-dextran	FPV in CEF with DEAE-dextran	FPV in ECE	FPV In CEF	Non vaccinated
0	0.4	0.3	0.3	0.4	0.3	0.2	0.4	0.3	0.3	0.3	0.1
1 week	0.9	1.2	1.1	1.3	1.1	1.3	1.4	1.3	1.3	1.2	0.2
2 weeks	1.6	1.4	1.5	1.3	1.8	2.0	2.0	1.7	1.6	1.7	0.1
3 weeks	2.3	2.1	2.3	2.0	2.4	2.1	2.2	2.4	2.4	2.0	0.1
4 weeks	2.1	1.9	2.0	1.8	2.3	2.0	2.0	2.2	2.1	1.9	0.2
5 weeks	1.9	1.8	1.9	1.8	2.0	1.9	1.9	1.8	1.9	1.8	0.1

Discussion

Usually viral vaccine producers hope to increase their production with a maximum possibility of cost reduction. One of the methods that helps in such purpose is to increasing virus infectivity titer in the vaccine product, so the main

goal of the present study was directed to increase the infectivity titer of FPV used for vaccine production and propagated on ECE and CEF using chemical enhancers like DEAE-Dextran and MgCl.

The cytotoxic effect of DEAE Dextran was evaluated in both CEF and ECE. The safe concentration used for DEAE-Dextran was 50ug/ml on CEF and 25ug/ml in ECE. Similar results were obtained by ***Kaplan et al (1967)*** who found that DEAE-Dextran (50 ug/ml) improved the susceptibility of cell culture to rabies virus, ***Zeneib Salama (2006)*** found that 50 ug/ml improved susceptibility of Vero cells to BEF virus, ***Eman et al (2011)*** found that 25ug of DEAE-Dextran is safe for ECE to increase the titer of propagated AI on ECE, ***Ayatollah et al (2007)*** mentioned that 50 ug/ml of DEAE-Dextran is the effective concentration increase CPV titer on Vero cell line and ***Soad et al. (1986)*** used the same concentration to increase FPV titer on chicken embryo rough cell (CER) and Vero cell line.

The cytotoxic concentrations of MgCl was determined on both CEF and ECE, it was found that the safe concentration is 30 ug/ml on CEF and 20 ug/ml on ECE. These result in parallel to ***Tsuctty and Tagaya (1970)*** who used 30 ug/ml of MgCl to increase the variola virus titer on monkey kidney cell line. Also ***Sahle*** et ***al (2002)*** used MgCl during propagation of n NDV on MDBK cell line.

By using DEAE-Dextran, the FPV titer increased gradually from 5.7 log_{10} TCID $_{50}$/ml in first passage reaching to 6.7 log_{10} $TCID_{50}$/ml by the 9th passage on CEF while on ECE the FPV titer increased gradually from 5.2 log_{10} EID $_{50}$/ml in first passage reaching 6.2 log_{10} EID_{50}/ml by the 8th passage compared to the non treated FPV which reach 6.2 log_{10} $TCID_{50}$/ml and 5.7 log_{10} EID_{50}/ml at 9th passage on CEF and ECE respectively. Similar results were obtained by ***Lee et al (1996)*** they reported an increase in the Retro virus titer by 3 fold by using DEAE-Dextran, ***Zeneib Salama (2006), Ayatollah et al (2008) and Olfat et al (2010)*** recorded the increase of BEFV, CPV and SPV titer, by one log in Vero cell line by using DEAE-Dextran. Also ***Eman et al (2011)*** reported the increase haemagglutinating activity (HA) with 2 log when 25ug/ml DEAE-Dextran used during AI virus inoculation on ECE.

The action of DEAE-Dextran could be explained on the basses of negatively charged surfaces of both cell and viruses, so the pretreatment of CEF cell monolayer with DEAE-Dextran would enhance the adsorption and uptake of the virus onto such cells ***(Tessy et al, 2004)***.

The titer of FPV treated with MgCl was more than that treated with DEAE Dextran reaching 7.5 $TCID_{50}$/ml on CEF by the 8th passage and 6.7 log_{10} EID_{50}/ml on ECE in 9th passages, similar results

obtained by ***Spizizen et al (1986)*** who found that using of divalent cations improved DNA transfection into cell culture as much as 100 times over the DEAE-Dextran method, ***Hussein et al (2003)*** mentioned that divalent cations in the outlaying medium of PPR virus elevated its titer on Vero cells comparing with conventional method.

Sahle et al (2002) reported an increase of NDV titer on MDBK cell line using MgCl. Also ***Tsuctty and Tagayab (1970)*** who mentioned that vaccinia virus titer increased one and half log using MgCl in Vero cell line. ***Graham and Van der Eb (1973)*** explained the mode of action of MgCl on the basis of divalent cations such as calcium and magnesium promote the uptake of DNA into cells (Transformation). Also ***Milan and George (1986)*** mentioned that the action of MgCl could be explained on the basis of enhancing adsorption and increasing virus release, both contribute to enhancement of virus titer and accelerate the onset of CPE in CEF. Similar explanation obtained by ***Abeer et al (2010)*** who reported that electrolytes potentially facilitate the adsorption and penetration of FMD to BHK cells by increasing virus attachment to the cell receptors.

The onset of CPE of FPV treated with DEAE- Dextran and MgCl appeared earlier than that of the non treated FPV on CEF by one day. Similar results were obtained by ***Zeneib Salama (2006)*** by using DEAE-Dextran with BEF on Vero cell line.

Protection percent of vaccinated chickens with enhanced FPV vaccines with DEAE-dextran and MgCl in both ECE and CEF were 96%, 92%, 96% and 92% respectively in these results are the same as obtained by ***Namaa (1998)*** who examined the protection rate for FPV vaccine propagated on both CEF and ECE.

The neutrization index of vaccinated chickens with field dose (3.7 $\log_{10}$TCID$_{50}$/ml) of non treated propagated FPV on ECE and CEF were the same as treated FPV with DEAE-dextran and MgCl propagated on ECE and CEF cells. Moreover the NI of the vaccinated chickens with treated FPV vaccines (10x field dose 4.7 $\log_{10}$ TCID$_{50}$/dose) giving slightly higher NI. The same results obtained by ***Aytollah et al (2007)*** who discrimated between the field dose and 10x field dose of treated CPV vaccine with DEAE-Dextran.

Recommendations

This study recommended the use of MgCl and DEAE-Dextran during production of FPV vaccine in order to obtain high virus titer leading to increase vaccine production and reduction of vaccine costs.

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محاولات لزياده القوه العياريه للقاحات جدرى الطيور المنتجه على البيض المخصب الخالى من المسببات المرضيه وخلايا الزرع النسيجى

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فى هذه الدراسه اجريت محاولات لزياده القوه العياريه لفيروس جدرى الطيور على كل من البيض المخصب الخالى من المسببات المرضيه وخلايا الزرع النسيجى الاولى المحضره من اجنه الدجاج باستخدام كلا من الديا ديكستران وكلوريد المغنسيوم وذلك للحصول على اعلى قوه عياريه من فيروس جدرى الطيور لا نتاج كميات كبيره من اللقاح.
حيث تم تمرير فيروس جدرى الطيور بعد اضافه مادتى كلوريد المغنسيوم والدياديكستران وكذلك بدون اضافتهما فى خلايا الزرع النسيجى واجنه البيض الملقح كلا على حده وقد لوحظ ظهور (CPE) اسرع على الخلايا الاوليه مع زياده القوى العياريه للفيروس على كل من البيض والخلايا فى حاله استخدام كلا من كلوريد المغنسيوم والدياديكستران.
تم تحضير لقاحات من جدرى الطيور بعد اضافه كل من مادتى كلوريد المغنسيوم والدياديكستران وبتقيم هذه اللقاحات المنتجه تم التاكد من نقاوتها وامنها وفاعليتها.
وبتقيم المناعه المكتسبه من اللقاحات المعالجه بالمادتين فى الدجاج باستخدام اختبار التعادل المصلى وجد ان معدل الاجسام المناعيه المكتسبه من اللقاحات المعالجه قريبه من معدل الاجسام المناعيه المكتسبه من اللقاح المستخدم بدون معالجه وذلك باستخدام الجرعه الحقليه او مضاعفتها بواحد log.
مما سبق يتضح ان استخدام مادتى الدياديكستران وكلوريد المغنسيوم بالتركيزات المحدده يؤدى الى زياده القوه العياريه لفيروس جدرى الطيور مما يزيد انتاج اللقاح و تقليل تكاليف انتاجه.