ISSN 1110-7219

COMPARATIVE STUDIES ON ATTENUATED AND INACTIVATED OIL EMULSION EGG DROP SYNDROME (EDS) VIRUS VACCINE PREPARED ON CHICKEN LIVER CELL CULTURE AND DUCK EGGS VACCINE

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ABSTRACT

The EDS-76 vaccines produced either in the allantoic cavity of embryonated duck eggs or in chicken liver cell cultures were comparatively studied as a living attenuated and inactivated oil emulsion vaccines. Live attenuated vaccine was prepared by propagation of EDS-76 virus in duck eggs followed by 30 passages on prepared chicken liver (CL) cells. The onset of CPE and best time of virus harvesting was determined for each virus passages on CL cells. 25th passages on CL cells, EDS virus loss its pathogenicity and gave 100% protection to the vaccinated chicks. Inactivated virus was prepared in either duck eggs or CL cells. Live attenuated and inactivated oil emulsion CL cell adapted EDS vaccines gave high immunity to the susceptible chicks based on lymphocyte blastogenesis assay, serum neutralization test, HI and challenge test as well as the inactivated duck eggs oil emulsion vaccine. The CL cells prepared vaccine gave 100% protection to the susceptible chicken when kept at 4°C for 4 months.

INTRODUCTION

The egg drop syndrome (EDS) virus was isolated for the first time in **1976** by **Van Eck et al.** at the Buxton Conference on Avian Adenoviruses and Infectious Bronchitis and termed "egg drop syndrome".

In Egypt EDS virus isolated for the first time from duck farms (**Hamouda, 1988**) and from chicken farms by **Ahmed (1995)**.

EDS disease affects laying hens cause a sudden and frequently drop in egg production with laying of soft shelled eggs (**Holmes et al., 1989**) which persist for 4-10 weeks (**Ahmed, 1995**).

Zsak et al. (1982) mentioned that in chicken liver cells, peak virus and intracellular HA titers

were reached after 48 hours and peak extracellular HA titers were seen after 72 hours.

Pfirschke (1989) reported that embryonated chicken liver cell culture has proved to be an appropriate and sensitive substrate for propagation of virus of infectious laryngotracheitis (ILT), infectious bronchitis (IB), infectious bursal Disease (IBD) and egg drop syndrome virus of fowl.

Bragg et al. (1991) found that a cytopathic agent was subsequently isolated in chicken embryo liver cell cultures and identified as EDS virus by haemagglutination inhibition and neutralization test.

Swain et al. (1993) found that EDS-76 virus replicated best to the highest titre in chicken embryo liver cells and less in duck embryo liver cells and duck embryo fibroblast cells. The cytopathic effect in chicken liver cells was marked by the presence of round and refractile cells and detachment of cells from the glass surface.

Kaur et al. (1997) stated that immune response to live and inactivated EDS virus can be detected by neutralizing antibody response and challenge reaction.

The aim of this present work is the comparison of the immune response of the prepared living attenuated and inactivated vaccine either CL cells propagated in CL cells or in duck eggs vaccine.

MATERIAL AND METHODS

1-Chicks:

Susceptible 21-days old Hubbard chicks were used for vaccine evaluation.

2-Virus strain:

EDS-76 virus strain supplied by the Central Veterinary Laboratory, Weybridge, England.

3-Embryos:

- One day old SPF chicks were used for preparation of chicken liver cell cultures supplied by **Pfirschke (1989)**.
- Embryonated duck eggs. They were obtained from United Company for Poultry Production and used for propagation and titration of EDS-76 virus.

4-Cell cultures media, reagents and solution:

4.1. Minimum Essential Medium (MEM):

It was used as growth medium with 10% newborn calf serum and maintenance medium with 2-3% newborn calf serum in pH 7.2. It was supplied by Sigma.

4.2. Preparation of inactivated vaccine:

EDS virus was inactivated with 0.1% formalin and emulsified with paraffin oil. The prepared vaccines were tested for sterility, potency and challenge according to **Lee-Amt and Hopkins (1982)**.

5. Methods:

5.1. Virus titration:

It was carried out according to **Pedro and Graham (1980)**. The virus titre was calculated according to **Reed and Muench (1938)**.

5.2. Serum neutralization test:

According to the method described by **Rossiter et al. (1985)**.

5.3. Haemagglutination inhibition test (HI):

It was carried out according to **Anon (1971)**.

5.4. Lymphocyte blastogenesis assay:

It was applied according to **Lee (1984)**.

RESULTS & DISCUSSION

EDS-76 is an infectious viral disease of paramount economic importance to the farmers (**Van Eck et al., 1976**) characterized by drop in egg production quantity and quality (**McFerran et al., 1978**).

Killed vaccines as well as live vaccines are being used for the prevention of clinical disease in birds (**Kaur et al., 1997**).

Humoral antibody response has been demonstrated to EDS-76 infection and vaccination. Recently cell mediated immunity response has also been demonstrated following EDS-76 virus inoculation (**Kumar et al., 1989**).

Embryonic chicken liver cell culture has proved to be an appropriate and sensitive substrate for propagation of egg drop syndrome virus (**Pfirschke, 1989**).

Table (1) shows the infectivity titre of the 3 passages of original propagated and titrated in embryonated duck eggs that reached to 10^6 EID_{50}/ml.

Dealing with results in table (2) propagation of the original EDS virus for 30 serial passages on chicken liver cell cultures and observing the start of CPE (round and refractile cells and de-

tachment of these cell from the glass surface) and the best time of harvesting indicated that the virus titre increase to the beak and reached to 10^{12} $TCID_{50}$/0.1 ml after 10 passages.

This result agree with those obtained by **Swain et al. (1993)** who found that the EDS-76 virus replicated best in primary chicken embryo liver cells and CPE can be observed by 24-48 hours after virus inoculation, and agree also with **Calnek et al. (1997)** who found that the virus was rapidly adapted to chicken liver cell cultures producing optimum titre of 10^7 $TCID_{50}$/ml at 7^{th} passage within 5^{th} day post inoculation.

Tables (3 and 4) shows that passage 25^{th} was completely safe and protective to chickens 21 day old vaccinated by 1.0 ml I/M of attenuated virus that observed for 21 days post inoculation and then challenged by virulent EDS-76 virus and kept under observation for 15 days after challenge.

From this results the 24^{th} passage of EDS virus on chicken liver cell gave a complete attenuated live protective virus that could be use for preparation of attenuated and formalin inactivated oil emulsion EDS vaccines which used in this study in comparison with the inactivated embryonated duck egg propagated EDS virus vaccine.

The final and main objective of this study was to prepare potent live attenuated and inactivated EDS-76 vaccines on CL cells and evaluate their efficacy in susceptible chickens in comparison with the local embryonated duck eggs prepared vaccine. The prepared vaccines were sterile as clear in table (5).

The efficacy of the different prepared vaccines was tested to determine the level and duration of cell mediated immune response for each of the investigated vaccines as mentioned in table (6). Antibodies were monitored in sera collected from vaccinated and non vaccinated birds by HI and SNT till 12 weeks post vaccination. the immune response was measured in table (7).

Tables (7, 8) show the peak of SNT and HI value from the 4^{th} to 12^{th} weeks post vaccination with live attenuated and from 4 to 12 weeks with inactivated CL cell vaccines while it was 6 to 12 weeks in duck eggs inactivated vaccine. That is agree with **Khalaf (1981)** who found that the neutralizing and haemagglutination inhibition antibodies in blood serum of vaccinated chicks give peak titres in between 7 and 12 weeks post vaccination. This result has been reported by **Phillips (1973) and Adu et al. (1989)**.

Table (9) indicated that after challenge test the three prepared vaccines (live attenuated, inactivated CL cell cultures vaccines and the embryonated duck eggs inactivated vaccine) gave 100% protection for three serial months post challenge with virulent EDS-76 virus.

The keeping quality of the prepared vaccine was tested for 4 months in -20°C and 4°C for live

attenuated and both inactivated vaccine respectively as shown in table (10) which clear that they gave 100% protection percent. As mentioned by **Rhee et al. (1987)** the vaccine afforded immunity as long as six months. From the previous results we could conclude that the successful trials of propagation and attenuation of EDS-76 virus in CL cell culture, it is rapid, specific, sensitive and reduce the probability of contamination as in the EDS-76 virus harvested from commercial duck eggs that collected from different sources that can carry different contaminants as bacteria, fungus and mycoplasma.

Table (1) Infectivity titre of EDS-76 virus propagated in embryonated duck eggs

No. of passages	log_{10} EID_{50} / ml
1	5
2	5

Table (2) Propagation and titration of EDS-76 virus propagated on chicken liver cell cultures (CL)

No. of passages	time of CPE appeared post inoculation (hours)	time of harvestation post inoculation (days)	log_{10} $ICID_{50}$ / ml
1	72	5	3
5	48	4	7
15	24	2	12
20	24	2	11
25	24	2	12
30	24	2	12

CPE = cytopathic effect

Table (3) Experimental infection of 21 days old chicks with EDS-76 virus propagated on chicken liver cells (attenuated)

No. of passages	No. of chicks used	No. of dead chicks	mortality percent	No. of contact control not challenged	No. of dead contact control chicks
1	10	10	100	3	3
5	10	10	100	3	3
10	10	2	20	3	3
15	10	4	40	3	0
20	10	2	20	3	0
22	10	2	20	3	0
23	10	4	40	3	0
24	10	0	0	3	0
25	10	0	0	3	0
30	10	0	0	3	0

Table (4) Protection efficiency of chicken inoculated with different EDS-76 virus passages on chicken liver cells after challenging test

No. of passages	No. of challenged chicks	No. of dead chicks	Morbidity %	Mortality %	PM lesions	No. of challenged control chickens	No. of dead control chicks after challenge
10	8	4	50	50	typical EDS lesion	3	3
15	6	2	30	30	typical EDS lesion	3	3
20	8	4	40	40	typical EDS lesion	3	3
22	8	2	25	25	typical EDS lesion	3	3
23	6	0	0	0	-	3	3
24	10	1	10	10	typical EDS lesion	3	3
25	10	0	0	0			
27	10	0	0	0	-	3	3
29	10	0	0	0			
30	10	0	0	0	-	3	3

Table (5) Sterility of the prepared EDS vaccines

Media	Living attenuated EDS CL cells propagated vaccine	inactivated EDS oil emulsion vaccine	
		CL cells propagated vaccine	embryonated duck eggs vaccine
Nutrient agar media	NC	NC	NC
Thioglycollate broth	NT	NT	NT
Sabauraud's glucose agar	NC	NC	NC
Grey media	NC	NC	NC

NC = No colonies appeared on used medium.
NT = No turbidity appeared on used broth.

Table (6) Results of cell mediated immune response of chickens post vaccination with prepared vaccines using lymphocyte blastogenesis assay

Groups No.	Type of vaccines used	weeks post vaccination			
		1	2	3	4
1	live attenuated EDS-76 propagated on CL cells	0.231	0.694	0.375	0.301
2	inactivated oil emulsion EDS-76 propagated on CL cells	0.252	0.755	0.357	0.298
3	inactivated oil emulsion EDS-76 propagated on duck eggs	0.116	0.175	0.122	0.090
4	control non vaccinated	0.037	0.034	0.035	0.035

Table (7) Log_2 mean neutralizing antibody titers of sera from vaccinated chickens with different prepared vaccines

Group No.	Type of vaccines used	weeks post vaccination											
		1	2	3	4	5	6	7	8	9	10	11	12
1	live attenuated EDS-76 on CL cells	11	10	10	12	11	11	12	12	11	12	12	12
2	Inactivated oil emulsion EDS-76 on CL cells	11	5	10	12	12	12	12	10	12	12	12	11
3	Inactivated oil emulsion EDS-76 on duck eggs	4	6	5	7	8	11	7	11	12	12	12	12
4	Control non vaccinated	0	0	0	0	0	0	0	0	0	0	0	0

Table (8) Mean HI antibody titers (log_2) of sera from chickens vaccinated with different prepared EDS-76 vaccines

Groups No.	Type of vaccines used	weeks post vaccination											
		1	2	3	4	5	6	7	8	9	10	11	12
1	live attenuated EDS-76 on CL cells	0	4	6	6	9	8	10	7	10	10	7	7
2	Inactivated oil emulsion EDS-76 on CL cells	7	7	6	10	9	7	6	6	7	5	6	6
3	Inactivated oil emulsion EDS-76 on duck eggs	9	10	9	9	8	8	8	7	7	7	7	7
4	Control non vaccinated	0	0	0	0	0	0	0	0	0	0	0	0

Table (9) Rate of protection of prepared EDS-76 vaccines

Group No.	Type of vaccines used	1st month			2nd month			3rd month		
		No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %
1	live attenuated EDS-76 on CL cells	5	5	100	5	5	100	5	5	100
2	Inactivated oil emulsion EDS-76 on CL cells	5	5	100	5	5	100	5	5	100
3	Inactivated oil emulsion EDS-76 on duck eggs	5	5	100	5	5	100	5	5	100
4	Control non vaccinated	5	0	0	5	0	0	5	0	0

Table (10) Keeping quality of prepared EDS-76 vaccines

Group No.	Type of vaccines used	Keeping temperature	Duration of potency (months)							
			1st		2nd		3rd		4th	
			S	%	S	%	S	%	S	%
1	live attenuated EDS-76 on CL cells	- 20 °C	5/5	100	5/5	100	5/5	100	5/5	100
2	Inactivated oil emulsion EDS-76 on CL cells	+ 4 °C	5/5	100	5/5	100	5/5	100	5/5	100
3	Inactivated oil emulsion EDS-76 on duck eggs	+ 4 °C	5/5	100	5/5	100	5/5	100	5/5	100
4	Control non vaccinated		0/3	0	0/3	0	0/3	0	0/3	0

S = survived chickens. % = protection %.

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الملخص العربى

دراسات مقارنة على لقاح حى مستضعف ولقاح زيتى ميت ضد الڤيروس المسبب لمرض تدنى البيض فى الدواجن المحضر على خلايا الكبد من كتاكيت خالية من المسببات المرضية واللقاح المحضر على بيض البط

نانسى بطـرس روفائيـل

معهد بحوث الأمصال واللقاحات البيطرية - العباسية

تم تحضير خلايا الزرع النسيجى (CL) من كتاكيت خالية من المسببات المرضية واستخدمت كبديل لإنتاج لقاح حى مستضعف وآخر ميت للڤيروس المسبب لمرض تدنى البيض فى الدجاج، وقد تم تعيين الوقت المناسب من التمريرة رقم (٢٤) للحصول على الڤيروس الحى المستضعف ذو أعلى قوة عيارية لعمل هذه اللقاحات واستخدم الفورمالين لتثبيط الڤيروس لعمل اللقاح الميت، وقد تم عمل دراسة مقارنة بين اللقاحات المحضرة على خلايا الزرع النسيجى (CL) واللقاح الزيتى الميت المحضر محلياً على بيض البط المخصب لمدة ١٢ إسبوع وبقياس مستوى المناعة باستخدام تجارب سيرولوجية مختلفة (HI-SNT) وبقياس المناعة الخلوية وأيضاً عمل إختبار التحدى بالڤيروس الضارى للكتاكيت المحصنة بالأنواع المختلفة من اللقاحات تحت الدراسة وثبت قدرتها المناعية كما تم تعيين مدى إستمرار الكفاءة المناعية Keeping quality للقاحات المحضرة على خلايا الزرع النسيجى بعد حفظها واللقاح الزيتى الميت فى ٤ م واللقاح الحى المستضعف فى - ٢٠م لمدة ٤ شهور.