TRIALS FOR PREPARATION OF INACTIVATED EGG DROP SYNDROME VIRUS VACCINE ADAPTED ON SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS (SPF-ECE)

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Abstract

Egg drop syndrome – 76 (EDS-76) virus was propagated for three passages on embryonated duck eggs followed by thirty passages on C.E.F cell culture, then, for ten passages on SPF embryonated chicken eggs. The titer and HA activity of the 10^{th} passage was $10^{9.5}$/ml and 2^{10}/ml successively. Inactivation of passage number 10 of propagated virus on SPF-ECE with formaline was adjuvanted with paraffin oil. The prepared vaccine was compared with inactivated oil emulsion EDS76 prepared on embryonated duck eggs for sterility, safety and potency. The potency test was performed by measuring the cellular and humoral immune response. The efficiency of the prepared vaccine was estimated up to six months.

INTRODUCTION

EDS virus has become a major cause of lost egg production throughout the world. It is caused by an adenovirus. The disease is characterized by the production of thin-shelled or shell-less eggs (Van Eck *et al.*, 1976).

The virus appeared to be transmitted vertically through the egg. The virus often remained latent until birds were approaching peak egg production (Calnek, 1978).

An oil adjuvant inactivated vaccine is widely used to giving good protection against clinical EDS (Christensen, 1998).

The birds were vaccinated between 14 and 16 weeks of age (Lee and Hopkins, 1982). HI antibody response can be detected by the 7^{th} day after vaccination with peek titers between the 2^{nd} and 5^{th} weeks. The vaccinal immunity lasts at least one year (Baxcndale *et al.*, 1980, Khalaf *et al.*, 1982).

The aim of the present study is directed to the preparation and evaluation of inactivated EDS vaccine on SPF embryonated chicken eggs.

MATERIALS AND METHODS

1. **Chicks**: two hundred susceptible 21-days old Hubbard chicks were used for vaccine evaluation.

2. **Virus strain**: EDS-76 virus strain was supplied by the Central Veterinary Laboratory, Weybridge, England.
3. **Embryos**
    a. Embryonated chicken specific pathogen free (SPF eggs were obtained from Ministry of Agriculture, Koum Osheim, Fayoum, Egypt). Nine to 10 days old SPF-ECE were used for preparing chicken embryo fibroblast cell culture according to Plowright and Ferris (1959) and modified by Osman *et al.* (1985) and also used for propagation of EDS-76 virus.
    b. Embryonated duck eggs: These were obtained from United Company for Poultry Production and used for propagation and preparation of inactivated oil EDS-76 vaccine.
4. **Cell culture media, reagents and solutations**
    a. Minimum Essential Medium (MEM): it was used as growth medium with 10% newborn calf serum and as maintenance medium with 2-3% newborn calf serum in pH 7.2. It was supplied by Sigma.
    b. Hank's balanced salt solution (HBSS): it was used for virus titration and was prepared according to Hank and Wallace (1949).
    c. Trypsin (1:250): it was used in primary cell culture preparation at a concentration of 0.25% according to Lennette (1964).

Methods

1. Adaptation and propagation of EDS-76 on chicken embryo fibroblast was carried out for thirty passages, and then, adapted and propagated to thirty and fifteen passages on SPF-ECE for 10 passages, and titration to each passage on SPF-ECE to detect HA activity to each passage was applied (Wo, 1995).
2. Preparation of different types of vaccines
    a. Inactivated oil emulsion EDS-76 on SPF-ECE.
    b. Inactivated oil emulsion EDS-76 on embryonated duck egg was applied according to Rozhest Vensk (1984).
3. Vaccination of chicks
    a. Each chick was inoculated with 0.5 ml of the prepared inactivated vaccine by s/c route .
    b. Safety test

        Each chick was inoculated with double dose (1 ml) of the prepared inactivated vaccine by s/c route.

The previous vaccines were tested for sterility according to OIE and for immunological effect with serum neutralization test according to (Rossiter *et al.*, 1985),and for Haemagglutination inhibition test HI according to (Anon, 1971), lymphocyte blastogenesis assay (Lee, 1994), protection percent and keeping quality (Lee and Hopkins, 1982).

RESULTS

Table 1. Propagation and titration of EDS-76 virus in SPF embryonated chicken eggs.

No. of passages	Log_{10} EID_{50}/ml	HA (log_2)
1	3.50	3
2	4.10	3
3	4.10	5
4	6.10	6
5	8.50	7
6	8.50	8
7	7.80	9
8	9.00	10
9	9.30	10
10	9.50	10

Table 2 . Sterility of the prepared EDS vaccine.

Media	Inactivated oil emulsion	
	Embryonated SPF propagated vaccine	Embryonated duck eggs vaccine
Nutrient agar media,	NC	NC
Thioglycolate broth	NT	NT
Sabauraoud's glucose agar	NC	NC
Grey media	NC	NC

NC = no colonies NT = no turbidity

Table 3 . Lymphocyte blastogenesis of chicks vaccinated with the prepared vaccines.

Group	Type of vaccines used	Weeks post-vaccination		
		1st	2nd	3rd
1	Inactivated oil emulsion EDS-76 propagated on SPF embryonated chicken eggs	0.72325	0.93325	0.96125
2	Inactivated oil emulsion EDS-76 on embryonated duck eggs	0.57320	0.84520	0.72340
3	Control non-vaccinated	0.18550	0.19200	0.18150

Table 4. Mean HI antibody titers (log2) of sera from chickens vaccinated with different prepared EDS-76 vaccines (Duration of immunity of prepared EDS vaccines).

Group	Types of used vaccines	Weeks post-vaccination																	
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24
1	inactivated oil emulsion EDS-76 on SPF embryonated chicken eggs	6	7	7	8	9	9	10	10	10	11	11	11	10	10	9	9	8	8
2	Inactivated oil emulsion EDS-76 on duck eggs	9	10	9	10	10	10	10	9	9	9	9	8	8	8	7	7	7	7
3	Control non-vaccinated	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. Log2 mean neutralizing antibody titers of sera from vaccinated chicks with different vaccines.

Group	Types of used vaccines	Weeks post-vaccination																	
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24
1	Inactivated oil emulsion EDS-76 on SPF embryonated chicken eggs	8	8	8	9	10	10	10	11	11	11	12	12	12	11	10	9	9	9
2	Inactivated oil emulsion EDS-76 on duck eggs	4	4	8	6	11	12	12	12	12	12	12	12	12	12	11	10	10	10
3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Rate of protection for the prepared EDS-76 virus vaccines.

G	type of used vaccines	1st month			2nd month			3rd month			4th month		
		No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %	No. of challenged	Survived	Protection %	No. of challenged	Survived	Protection %
1	inactivated oil emulsion EDS-76 on SPF embryonated chicken eggs	5	5	100	5	5	100	5	5	100	5	5	100
		5	5	100	5	5	100	5	5	100	5	5	100
2	Inactivated oil emulsion EDS-76 on duck eggs	5	5	100	5	5	100	5	5	100	5	5	100
3	Control non-vaccinated	5	0	0	0	0	0	5	0	0	5	0	0

Table 7. Keeping quality of prepared EDS-76 virus vaccines (months).

Group	Type of vaccine used	Temp of storage	Protection %							
			1st month		2nd month		3rd month		4th month	
			Survived chickens	Protection %	Survived chickens	Protection %	Survived chickens	Protection %	Survived chickens	Protection %
1	inactivated oil emulsion EDS-76 on SPF embryonated chicken eggs	4 °C	5/5	100	5/5	100	5/5	100	5/5	100
2	Inactivated oil emulsion EDS-76 on duck eggs	4 °C	5/5	100	5/5	100	5/5	100	5/5	100
3	Control -	-	0/2	0	0/2	0	0/2	0	0/2	0

DISCUSSION

The aim of the present study was directed to prepare a safe and protective inactivated SPF embryonated chicken egg vaccine against EDS-76, and comparing it with inactivated oil EDS prepared on duck embryonated eggs. The scheme used for preparing the vaccine included the propagation of the adapted EDS-76 virus on CEF cells for 10 passages on SPF-ECE.

Table 1 showed that the titer of the propagated EDS virus on SPF-ECE gradually increased the 1^{st} passage$10^{3.5}$ and reached to maximum in the 10^{th} passage$10^{9.5}$. These results are in agreement with those obtained by Wo (1995).

Table 2 showed that both vaccines were tested for sterility and proved to be free from any contaminants. A high value of lymphocyte blastogenesis for three weeks post-vaccination was shown with the prepared vaccines when compared with control (Table 3). These results were in agreement with Umesh-Kumar *et al.* (1989).

Table 4 showed the evaluation of the humoral immune response HI of chicken vaccinated with the prepared vaccine when compared with vaccine prepared on duck embryonated eggs. It was clear that high titer value of haemagglutination inhibition titer appeared in vaccinated groups for 24^{th} weeks. It was shown that HI titer in chicken vaccinated with ECE vaccine had increased gradually and reached its maximum 2^{11} in 10, 11, 12, weeks and still being high 2^{8} to the 24^{th} week, while , HI value of vaccinated chickens with embryonated duck egg vaccine had reached its maximum 2^{10} in 4^{th} -7^{th} till reached 2^{7} in 24^{th} week. These results were in agreement with Chen Wen Xian *et al.* (1996).

Table 5 showed that there was negligible difference of neutralizing antibody titre in chicken vaccinated with different vaccines, while, the control group was zero. This result agreed with (Kaur *et al*, 1997).

Table 6 showed that chicks vaccinated with prepared vaccines gave high protection percentage reaching 100% when vaccinated chicks were challenged with virulent EDS virus after 3 weeks, while, controls did not show any protection. This result agreed with Cook (1983).

By discussing the keeping quality of the prepared inactivated EDS virus vaccines when stored at 4 $^{\circ}$C (Table 7), it was clear that the vaccine was stable and potent for a period of 6 months as protection reached 100%. This result agreed with Khalaf (1981) and Picault *et al.* (1982) who reported that the antigen released over a long period with high immune response after one dose.

We could conclude that the 10^{th} passage of EDS virus on SPF-ECE and the preparation of inactivated oil emulsion vaccine gave safe, potency and immunogenicity. So, from the previous results, we can conclude that the inactivated oil emulsion vaccine prepared on embryonated chicken egg is safe, potent and immunogenic. So, we could use the SPF-ECE instead of embryonated duck eggs.

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محاولة لتحضير لقاح مثبط ضد فيروس مرض تدني البيض في الدواجن على أجنة البيض الخالي من المسببات المرضية

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تم محاولة تمرير فيروس مرض تدني البيض المستضعف على خلايا أجنة الكتاكيت، عشر تمريرات على أجنة البيض الخالي من المسببات المرضية، وقد تم قياس القوة العيارية وقوة التلازن الدموي بعد تمريرة على حدة ، وقد تم تحضير لقاح ميت من التمريرة العاشرة باستخدام الفورمالين وتم حقنه في الكتاكيت القابلة للعدوى وقياس المناعة الخلوية واستخدمت التجارب السيرولوجية (إختبار التعادل المصلي وإختبار منع التلازن الدموي) لقياس المناعة للقاحات المختبرة على الكتاكيت (لقاح زيتي ميت على أجنة بيض الدجاج ولقاح زيتي ميت على أجنة بيض البط). كما تم إختبار القدرة المناعية للدجاج المحصن باستخدام تجربة التحدي بالفيروس الضاري، وقد تم حفظ اللقاح الميت في درجة ٤ م° لمدة ستة أشهر متتالية وإختبار قوة الصد للكتاكيت المحصنة بكل منها .

و توصي الدراسة السابقة بأنه يمكن استخدام أجنة البيض الخالي من المسببات المرضية في تمريرفيروس مرض تدني البيض في الدواجن بدلاً من استخدام أجنة بيض البط كما توصي بتحضير لقاح مثبط علي أجنة البيض الخالي من المسببات المرضية حيث أثبت اللقاح المحضر أنه آمن ، ذو كفاءة عالية و يعطي رد فعل مناعي يكفي لحماية الكتاكيت المحصنة مدة لا تقل عن ستة أشهر .