Thermotolerant Effect Of Trehalose Dehydrate On The Efficacy Of Fowl Pox Vaccine Prepared In Spf Eggs

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ABSTRACT

In this study, trehalose dihydrate was used in two concentrations(2.5 % and 5%) alone or in combination with lactoalbumin hydrolysate to prepare different formulae of fowl pox vaccine. The prepared vaccines were tested for their stability either in the lyophilized or reconstituted form.

The obtained results showed that use of 5% trehalose either combined with lactalbumin hydrolysate or alone proved vaccine efficacy for a period of 15 days at 37°C and 8, 9 hours at 45°C more than 2.5 % trehalose concentration (12 days at 37°C and 5, 6 hours at 45°C).

Vaccination of susceptible birds with different formula and monitoring the antibody level using serum neutralization showed higher antibody response were recorded using the formulae containing 5% trehalose. Challenging the vaccinated birds (with any formula) using virulent fowl pox virus showed protection of all birds vaccinated with fowl pox vaccine.

INTRODUCTION

Fowl pox is a slow spreading viral disease of poultry that occurred worldwide. The fowl pox virus is highly infectious for chickens, turkeys and pigeons. It is a DNA virus of the genus *Avipoxvirus* of the Family Poxiviridae *(1).*

Vaccination is one of the most effective tool for preventing viral diseases where attenuated fowl pox live virus vaccines are widely used throughout the world. These vaccines must be stored, transported and applied appropriately as recommended by the manufacturers. They must be protected from deterioration by high temperatures and inproper handling *(2).*

An object of the present study is to provide an improved stabilizer to have a prolonged store stability with a diminished reduction in titre. Trehalose dihydrate is a stable, chemically non-reactive disaccharide, yet exhibit flash solubility on hydration *(3)* and this property is particularly useful when reconstituting dried vaccines.

This study was planned to trial of improvement the quality of the used live fowl pox vaccine, through the incorporation of trehalose dihydrate to maximize the vaccine thermostability and minimize its titre loss .

MATERIAL AND METHODS

1. Experimental birds

Ninety apparently healthy 3 weeks-old susceptible chickens to fowl pox virus were used in this study. The birds were calssified into 6 equal groups (15 birds each) and used for vaccination, and challenge infection.

2. Fertile chicken eggs

Fertile specific pathogen free (SPF) chicken eggs, 9-11 days-old were obtained from Koam-Osheim, El-Fayoum SPF Farm, Egypt; were used in the titration of prepared fowl pox vaccines and serum neutralization test.

3. Vaccine preparation

Fowl pox seed virus was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI), with a titre of $10^{8.1}$ EID_{50}/ml. It was used for preparation of different batches of fowl pox vaccines.

4. Challenge pox virus

A local virulent fowl pox strain (obtained from Pox Department, VSVRI) with a titre of 10^{7} EID_{50}/ml was used for challenging.

5. Stabilizers

5.1. Lactalbumin hydrolysate

An enzymatic hydrolysate of lactalbumin was obtained from DIFCO Laboratories, USA. It was used as 5% concentration in preparation of different stabilizers.

5.2. Sucrose

It was obtained from Sigma Chemical Company, used as 2.5% concentration in preparation of lactalbumin sucrose stabilizer (the currently used stabilizer).

5.3. Trehalose dihydrate

It was obtained from SERVA Electrophoresis Company and was used in two concentrations (2.5% and 5%) alone and in combination with lactalbumin hydrolysate.

5.4. Preparation of the stabilizers

Five formulae (F) of stabilizers were prepared as follows:

1. F1 (the currently used stabilizer) 5% lactalbumin hydrolysate (LA) and 2.5% sucrose (S) in double distilled water (DDW).
2. F2: 5% LA and 2.5 % trehalose (T) in DDW.
3. F3: 5% LA and 5% T in DDW.
4. F4: 2.5 % T in DDW.
5. F5: 5% T in DDW.

All the formulae were sterilized by autoclaving.

6. Serum samples

Serum samples were collected from all groups of birds before vaccination and weekly after vaccination for monitoring antibody response during the experiment.

7.Preparation of vaccine batches

Stabilizers were added to the attenuated live fowl pox virus at the ratio of 1:1. The prepared vaccines were distributed in sterile neutral vials as 2 ml of each vaccine/10 ml glass vial, then exposed to lyophilization.

8. Titration of fowl pox vaccines

Titration was applied using embryonated chicken eggs *(4)*, the titre of fowl pox virus vaccine was expressed by EID_{50}/ml *(5)*.

9. Thermal stability of the prepared fowl pox vaccines

Each of the five vaccine batches was divided into 3 divisions:

First division: was exposed to 37°C for 21 days and titrated every 3 days.

Second division: was reconstituted and exposed to 37°C for 24 hours and titrated.

Third division: was exposed to 45°C for 21 hours and titrated.

10. Immune response of susceptible chickens to prepared prewared vaccine

First division of fowl pox vaccines that exposed to 37°C for 21 days were tested by vaccination of 75 susceptible chickens (15 chickens for each formula) using wing web method *(6)*; "takes" observed a week post vaccination with keeping 15 chickens as non-vaccinated control. Serum samples were collected at weekly intervals for 3 weeks from all chickens.

11. Challenge test

Challenge test was applied for all vaccinated and control chickens with the virulent fowl pox virus using wing web route (6) 3 weeks post vaccination.

12. Serum neutralization test (SNT)

SNT was carried out in embryonated chicken eggs using serum samples collected from chickens *(4)* for antibody evaluation of prepared vaccine.

RESULTS AND DISCUSSION

Live virus vaccines are vulnerable to inactivation by high ambient temperatures in countries of underdeveloped Veterinary services. Problems are encountered in maintaining the "cold chain" from manufacture to the point of administration in remote, hot, rural areas *(7)*. To some extent, the problem has been alleviated by stabilizing agents, selection of vaccine strains that are inherently more heat stable and by packaging them in freeze-dried form for reconstitution *(8)*.

This study was directed to the use of trehalose as stabilizer for fowl pox vaccine.

Table 1 showed a comparison between the two used carbohydrates, sucrose and trehalose, the mean titre losses after lyophilization was 1.0 log_{10} EID_{50} in case of sucrose as stabilizer while it ranged from 0.3, 0.4, 0.6 and 0.7 log_{10} EID_{50} in case of trehalose .Was reported that during freeze drying and rehydration, the protective efficiency of carbohydrates was as follows: trehalose > maltose > sucrose ***(9)***. Trehalose found to be very effective in the stabilization of labile proteins during lyophilization and exposure to high temperatures in solution ***(10)***.

The different lyophilized fowl pox vaccines formulae were tested for thermal stability as shown in Tables 2, 3 and 4. Table 2 showed that the protectivity of the lyophilized vaccines stored at 37°C decreased daily in which F1 (current used vaccine) was protective till 9 days, F2 and F4 remained protective for 12 days while F3 and F5 extended for 15 day.

Table 3 showed that after keeping of the reconstituted fowl pox vaccine formulae at 37°C, F1 (currently used vaccine) did not exceed for 8 hours stable, while F2 and F4 with 2.5 % trehalose extended for 10 hours and 5% trehalose for F3 and F5 for 14 hours stable respectively.

Table 4 proved that the lyophilized vaccines F1, F2 and F4 were stable for 4, 5 and 6 hours respectively, when kept at 45°C; while F3and F5 lyophilized vaccines formulae that contain 5% trehalose for 8 and 9 hours respectively. The previous results (Tables 2-4) revealed that lyophilized fowl pox vaccines having 5% trehalose (F3 and F5) were more stable than that containing 2.5 % trehalose (F2 and F4); or containing 2.5 % sucrose (F1). These results were similar to those previously demonstred in other studies ***(11, 12)*** which showed that vaccines treated with 5% trehalose dihydrate and exposed to 37°C through 2 weeks revealed an excellent degree of virus protection.

As well, it was axiomatic to find out the immune response in susceptible birds to such fowl pox vaccines which exposed to 37°C for 21 days. The susceptible birds were inoculated with the different formulae of the vaccine by wing web route and observed for 7-10 days for evidence of "takes" which consist of swelling of the skin or a scab at the site of injection and indicated successful vaccination, beside immune response.

The obtained results (Table 5) showed significant higher neutralizing index (2.2 and 2.2 NI) for F3 and F5 than that of the current used vaccine (F1) with sucrose (1.9 NI) at 3 weeks post vaccination and showed high protection against challenge with the virulent fowl pox virus (Table 6). Thus an excellent observation must be recorded here; the exposure of trehalose-treated fowl pox vaccines to 37°C did not deteriorating to the integrity of the virus particles as evidenced by the provoked neutralizing antibody response which has been recorded by previous several studies ***(13-15)***. Trehalose dihydrate is a multifunctional carbohydrate that can improve storage stability of biological materials. It is a unique, naturally occurring dissacharide which does not brown when heated (i.e. heat stable), it does not promote bacterial growth and it is less attractive to moisture. If sufficient trehalose is present, it is able to replace the water molecules as they are lost by dehydration to form multiple external hydrogen bonds and thus maintain the secondary structure of proteins ***(16)***.

In conclusion, trehalose dihydrate 5% stabilizer is not only suitable for stabilizing lyophilized fowl pox virus but also used against heat inactivation during lyophilization, storage period and transportation post lyophilization, which can insure effective field vaccination despite the problems of transportation and hot weather.

Table 1. Effect of lyophilization on different stabilizer batches of fowl pox vaccine

Fowl pox vaccine formula	Incorporated stabilizer	Titration before lyophilization	Titration post lyophilization	Losses in titres log_{10} EID_{50}/ml
F1	5% LA/2.5% S	8.1	7.1	1.0
F2	5% LA/2.5% T	8.1	7.4	0.7
F3	5% LA/5% T	8.1	7.7	0.4
F4	2.5 % T	8.1	7.5	0.6
F5	5 % T	8.1	7.8	0.3

EID_{50} = Embryo Infective Dose fifty LA= Lactalbumin hydrolysate
S= Sucrose T= Trehalose dihydrate

Table 2. The titre of lyophilized vaccines kept at 37°C for 21 days (log_{10} EID_{50}/ml)

Time (day)	F1	F2	F3	F4	F5
0	7.1	7.5	7.7	7.6	7.8
3	6.2	6.4	6.9	6.5	7.1
6	5.1	5.8	6.3	5.9	6.5
9	4.0 *	5.0	5.8	5.0	6.0
12	3.3	4.5 *	5.0	4.5 *	5.1
15	2.8	3.6	4.2 *	3.7	4.3 *
18	1.5	2.5	3.5	2.5	3.7
21	1.2	1.7	2.9	1.8	3.0

* The least infectivity titre is 10^4 EID_{50}/ml (6)

Table 3. The titre of reconstituted vaccines kept at 37°C for 24 hours (log_{10} EID_{50}/ml).

Time (hour)	F1	F2	F3	F4	F5
0	7.1	7.5	7.7	7.6	7.8
2	6.6	7.0	7.4	7.2	7.5
4	6.0	6.2	6.8	6.5	7.0
6	5.5	5.7	6.5	5.9	6.7
8	4.5 *	4.9	5.9	5.0	6.0
10	3.8	4.1 *	5.3	4.4 *	5.5
12	2.5	3.5	4.6	3.8	4.9
14	2.1	3.0	4.0 *	3.1	4.2 *
16	1.5	2.4	3.1	2.5	3.5
18	-	1.7	2.5	1.9	2.9
20	-	-	1.9	-	2.2
22	-	-	-	-	1.8
24	-	-	-	-	-

*The least infectivity titre is 10^4 EID_{50}/ml *(6)*

Table 4. The titre of the lyophilized vaccines kept at 45°C for 12 hours (log_{10} EID_{50}/ml).

Time (hour)	F1	F2	F3	F4	F5
0	7.1	7.5	7.7	7.6	7.8
1	6.9	7.0	7.3	7.1	7.4
2	5.6	6.5	6.9	6.7	7.1
3	4.8	5.4	6.5	5.6	6.7
4	4.3 *	5.0	5.7	5.1	6.0
5	3.8	4.5 *	5.2	4.5	5.6
6	3.1	3.8	4.7	4.0 *	5.3
7	2.6	3.2	4.3	3.5	4.9
8	2.0	2.5	4.0 *	3.0	4.5
9	1.5	1.9	3.6	2.1	4.0 *
10	-	-	2.9	1.5	3.5
11	-	-	2.0	-	2.9
12	-	-	1.5	-	2.2

*The least infectivity titre is 10^4 EID_{50}/ml *(6)*

Table 5. Fowl pox neutralizing indices for birds vaccinated with the different formulae which exposed to 37°C for 21 days.

Bird group	Type of vaccination	Time post vaccination (week)		
		1	2	3*
I	Vaccinated with F1 (the current used vaccine).	0.4	1.6	1.9
II	Vaccinated with F2	0.4	1.5	1.9
III	Vaccinated with F3	0.5	1.8	2.2
IV	Vaccinated with F4	0.4	1.5	2.0
V	Vaccinated with F5	0.5	1.6	2.2
VI	Control non-vaccinated birds	0.3	0.4	0.4

*Challenge time with the virulent fowl pox virus 3 weeks post vaccination.
N.B. Neutralizing index $\geq$ 1.5 is considered protective *(17).*

Table 6. Results of challenge test with virulent pox virus in different groups of chickens (n=15).

Group No.	Type of vaccination	No. of positive reactors *	No. of negative reactors **	Protection percentage post challenge
I	F1	1	14	93 %
II	F2	1	14	93 %
III	F3	0	15	100 %
IV	F4	1	14	93 %
V	F5	0	15	100 %
VI	Non-vaccinated control	15	0	0 %

* (a) is non protected birds showing typical signs of pox infection.
** (b) is protected birds showing no pox lesions

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الملخص العربى

التأثير الحرارى للتريهالوز ديهيدرات على كفاءة لقاح جدرى الطيور المحضر على بيض خالى من المسببات المرضية

منال عوض، ألفت إسطفانوس نخلة ، شيرين سعيد سمير، سعاد محمد سليمان
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تريهالوز ديهيدرات واحد من أكفأ المثبتات (stabilizers) التى تعمل على زيادة الثبات الحرارى للقاحات الحية المستضعفة. وفى هذه الدراسة تم استخدام تريهالوز ديهيدرات بتركيزين (٢,٥%، ٥%) منفردين أو كل منهما مخلط مع لاكت البيومين لتحضير نماذج مختلفة من لقاح جدرى الطيور. وقد تم تخزين هذه النماذج فى درجات حرارة مختلفة (٣٧°م، ٤٥°م) لفترات متفاوتة سواء كانت مجفدة (lyophilized) أو سائلة (reconstituted).

وأثبتت النتائج ان استخدام التريهالوز كمثبت بنسبة ٥% بخلطه مع لاكت البيومين أو بمفرده زاد من كفاءة تخمل اللقاح (١٥ يوم عند درجة حرارة ٣٧°م ومن ٨ الى ٩ ساعات عند درجة حرارة ٤٥°م) بالمقارنة بنسبة تركيزه ٢,٥% (١٢ يوم عند درجة حرارة ٣٧°م ومن ٥ الى ٦ ساعات عند درجة حرارة ٤٥°م).

وقد أثبتت هذه الكفاءة بتحصين الطيور القابلة للعدوى بالنماذج المختلفة حيث أنه بتقييم مستوى الاجسام المضادة باستخدام اختبار التعادل المصلى وجد أن أعلى مستوى للأجسام المضادة سجل بالنماذج التى تحتوى على ٥% تريهالوز وايضاً بإجراء اختبار التحدى وجد أن هذه الطيور اكتسبت حماية ضد العدوى بجدرى الطيور.