STUDY OF INCORPORATION DEAE DEXTRAN DURING PRODUCTION OF LOCAL AVIAN INFLUENZA (AI) INACTIVATED VACCINE

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Abstract

Two experiments have been conducted to study incorporation of di-ethyl amino ethyl dextran (DEAE Dextran) during production of avian influenza (AI) inactivated vaccine.

The first was planned to study addition of DEAE Dextran with different concentrations (25μg, 50μg and 100μg/0.1ml) to the virus inoculum seed before egg inoculation. Obtained results revealed selection of 25μg DEAE Dextran/0.1 ml that resulted in increasing haemagglutinating activity (HA) with 2 log and virus titre (EID_{50}) with 3.2.

In the second experiment, the same concentrations were added and tested separately during vaccine preparation with two different adjuvants. Serum of vaccinated chickens with the vaccine to which 100 μg DEAE Dextran / 0.5 ml was added, developed satisfactory raise in antibody level in comparing to positive control groups which received the vaccine without any additives.

INTRODUCTION

Avian influenza (AI) is an extremely infectious disease caused by type A strain of influenza virus which is a serious threat to poultry industry, and having almost 100% fatality rate. Since February 2006 the highly pathogenic (HP) avian influenza (H5N1) had emerged as the cause of severe disease, high mortality in chickens on production farms and backyard based production of Egypt. By time, the disease had spread allover the country threatening the poultry industry and causing a great hazard to human (Aly *et. al.*, 2006).

Beside strict biosafety and biosecurity and the strict hygienic measures, the availability and use of effective vaccine can be a valuable tool in controlling outbreaks of AI (Trevor *et. al.*, 2004). The existence of large number of virus subtypes together with the known variation of different strains within a subtype, some isolates did not grow to a sufficiently high titre to produce adequately potent vaccines without costly prior concentration (Bankowski, 1985).

It is well known that biosafety and biosecurity and strict hygienic measures appear to be the first line of defense against AI, but, vaccination still the second line of defence.

Many attempts were made for vaccine preparation and production locally (Abd El-Wanis *et. al.*, 2008). Polycations, such as protamine and diethylaminoethyl dextran (DEAE-Dextran) have been used extensively to facilitate the uptake of infectious viral nucleic acid in tissues (Pagans and Vaheri, 1987). DEAE-Dextran could possibly act by complextion of virus particles allowing them to attach to cell surface more efficiently. Another theory is that the DEAE Dextran can act by binding to the cellular surface, thereby, creating a favourable ionic charge for virus attachment. Also, there is a corresponding enhancement effect for DEAE Dextran on the infectivity of intact virus, being noticed by Barahona and Hasson (1968) for Newcastle disease virus. The enhancement of DEAE-Dextran was not only for viruses uptake but also for the uptake of proteins and nucleic acids which directly are dependent on the molecular weight of DEAE Dextran (Warden and Thorne, 1969). The current study described a valuable support during avian influenza vaccine preparation and production.

MATERIALS AND METHODS

Seed virus: The low pathogenic (LP) A/Turkey/CA/209092/02 (H5N2) avian influenza (AI) virus was used as the vaccinal strain. The virus was provided by the United State Department of Agriculture (USDA, Ames) where it has previous approval as H5. AI virus seed stock used for inactivated vaccine. The original titre of the virus was $10^{9.5}$ EID_{50}/ml with HA 9 log_2.

Virus propagation: According to Garcia *et. al.* (1998), the working seed was diluted in sterile physiological normal saline pH 7.2 that, bout 10^3-10^4 EID_{50}/0.1ml DEAE-Dextran was added to the inoculum at a concentration of 25, 50 and 100 μg/0.1 ml, while positive control eggs were inoculated with the virus only into the allantoic cavity of 10 days old embryonated specific pathogen free (SPF) chicken eggs, then, incubated at 37°C for 5 days. Infected eggs were chilled before being harvested. The appropriate amount of penicillin, streptomycin and mycostatin were added to the fluid.

Virus titration: Obtained harvested virus from each inoculum group was titrated in embryonated SPF chicken eggs, and the titre was expressed after the calculation according to Reed and Muench (1938).

Virus inactivation: According to Office International des Epizootics (OIE) Publication Manual (2004), inactivation of the virus was carried out using formalin at a final concentration of 0.1% of the total volume. One sample from the inactivated virus was tested for safety in embryonated chicken eggs and two successive blind passages were carried out before it was considered safe.

Preparation of oil adjuvant (AI) vaccine: Water in oil emulsion vaccine was prepared according to Stone *et. al.* (1978) with both the oil soluble surfactant (Span 80) and the aqueous soluble surfactant (Tween 80) which were added to the oil ratio at 1:2. It was used and adjusted with DEAE-Dextran concentration of 25, 50, 100 μg/0.5 ml final product as an experimental batch.

Preparation of alhydragel AI vaccine: An experimental batch of inactivated alhydragel vaccine was kindly supplied by Newcastle Disease Department and DEAE-Dextran was added separately with a final concentration of 25, 50, 100 μg/0.5 ml final product.

DEAE-Dextran HCl: A solution of 0.1% Di-ethyl amino-ethyl Dextran MW: 500,000 Pharmacia Fine Chemicals, Sweden, was prepared at 0.25 M Tris-HCl buffer of pH 8.2. This was autoclaved and the pH was adjusted to 7.6-7.8. It was kept at room temperature until used with the appropriate suggested concentration of 25, 50 and 100 μg and used separately in the vaccine inoculum and during vaccine preparation (DEAE Dextran adjuvant vaccines) (Anderson *et. al.*, 1971).

Glycine (NH2-Ch2-COOH): A product of El-Nasr Pharmaceutical Company was used as 4.8gm/litre for oil adjuvant vaccine.

c. Sodium thiomersal 1/10000 was added as 1ml/litre.

Vaccination and vaccine efficacy studies: A total of 180 SPF chicks 21 days old were divided into nine groups each of 20 chicks and were employed as follows:

Groups 1, 2, 3 received oil emulsion inactivated AI vaccine with different concentrations of DEAE-Dextran.

Groups 4, 5, 6 received alhydragel inactivated AI vaccine with different concentrations of DEAE -Dextran.

Group 7 positive control for oil emulsion AI vaccine, which received AI vaccine without any additives.

Group 8 positive control for alhydragel AI vaccine without any additives.

Group 9 non-vaccinated control group.

Birds of 1-8 groups were vaccinated subcutaneously at the nap of the neck with the recommended dose 0.5ml. The prepared vaccine containing 10^9 EID50 before inactivation, blood samples were weekly collected from each group up to 6 weeks post-vaccination. Serum was separated and tested for detection of humoured immune response using haemagglutination test (HI) according to Anon (1971) using beta procedure.

RESULTS AND DISCUSSION

The influences of vaccine strain and antigen mass on the ability of inactivated avian influenza (AI) viruses to protect chickens are the most important issues that help in vaccine production.

Vaccination with the heterologous neuraminidase (N2) could be a useful tool for the control of H5N2 epidemic in poultry (Lee *et. al.*, 2007). Moreover, using heterologous vaccine allowed identification of infected chickens within the vaccinated groups.

According to Swayne (2005)**,** the AI virus strains selected for manufacturing inactivated vaccine have been based on low pathogenic viruses that have homologous haemagglutinin protein (H5).

As the quantity of AI antigen in vaccine increased, all parameters of protection improved (Swayne, 1999). One of the outstanding effects of DEAE-Dextran is its ability to enhance viral infectivity in the cell, enhance the uptake of protein and nucleic acid by cells which allow penetrating more rapidly and superior transference.

Regarding the first experiment, addition of three different concentrations of DEAE Dextran to AI inoculum seed as shown in Table 1, revealed that addition of 25 μg/ml or 50 μg/ml of DEAE Dextran to the inoculum resulted in the same increase of virus haemagglutinating (HA) activity by 2 log2 (10) and the virus titre by 3 log10 (10.5) EID50/ml compared to harvested allantoic fluid of positive control eggs inoculated without any additives (8 log2 and 7.3 EID50/0.1ml). On the other hand, addition of 100μg/ml showed slight increase in (HA) activity and EID50 of the virus (1 log2 "9" and 2.4 log10 "$10^{9.7}$" EID50/ml) compared to the positive control harvested fluid. These results are in agreement with those obtained by Mansour (1995) and Madkour *et. al.* (2003).

The results of experiment two as illustrated in Table 2 revealed that addition of DEAE Dextran to an experimental batch of inactivated AI vaccine with two different adjuvants (alhydragel and oil emulsion) with the three choiced concentrations showed that, addition of 100μg/ml of DEAE-Dextran to oil adjuvanted vaccine showed the highest level in antibody titre by 2.0 log2 (9.5) in comparison to positive control group (7.5) at the 7^{th} week post-vaccination. On addition of the same concentration (100μg/ml) to alhydragel inactivated vaccine, it showed an increase in the antibody titre by 1.6 log2 (8.6) compared to positive control group (7.0) at the 7^{th} week post-vaccination.

On the other hand, addition of the other two concentrations of DEAE Dextran (25μg/ ml and 50μg/ml) it showed an increase in the geometric antibody titre by (0.4) and (0.6) log2 for alhydragel vaccine and by (1.6), (1.4) for oil adjuvanted vaccine.

Both results were explained by Wittman *et. al.* (1970) who suggested that the adjuvanted activity of DEAE Dextran could be attributed to a membranous effect on the immunocompetent cells to become antibody producer.

In conclusion, obtained results showed that 25 or 50 μg/0.1ml of DEAE-Dextran were of great benefit when added to AI virus seed inoculum, and 100 μg/ml when added to the inactivated vaccine could be an acceptable additive to increase the antibody response of vaccinated birds for eight weeks post-vaccination.

Table 1. Results of titration of the harvested amnioallantoic fluid after addition of DEAE-Dextran to AI inoculum.

DEAE Dextran concentration μg / 0.1ml inoculum	HA Unit (Log_2)	Virus titre EID_{50}/ml
0	8	7.3
25	10	10.5
50	10	10.3
100	9	9.7

Table 2. The antibody titre of haemagglutination inhibition test (HI) of vaccinated chicken groups

DEAE-Dextran Concentration	Groups	Weeks post-vaccination (Mean log2 HI titre)							
		1st	2nd	3rd	4th	5th	6th	7th	8th
25	1	2.0	2.8	4.3	5.8	7.0	8.9	9.2	9.0
50	2	2.5	2.5	4.8	6.5	7.3	8.1	10.0	-
100	3	2.5	3.0	5.1	6.6	8.1	9.5	10.8	10.8
25	4	2.1	2.4	3.5	5.0	6.4	7.5	8.2	8.0
50	5	2.2	2.3	4.1	5.8	7.0	8.4	9.0	9.0
100	6	2.2	2.2	4.5	6.1	7.0	8.6	9.1	9.0
0	7	2.5	2.5	3.6	5.0	5.7	6.9	7.7	8.0
0	8	2.3	2.2	4.2	4.8	6.0	7.0	8.0	8.5
0	9	0	0	0	0	0	0	0	0

Group (1): Vaccinated with 0.5 ml oil adjuvanted vaccine + 25μg/ml DEAE Dextran
Group (2): Vaccinated with 0.5 ml oil adjuvanted vaccine + 50μg/ml DEAE Dextran
Group (3): Vaccinated with 0.5 ml oil adjuvanted vaccine + 100μg/ml DEAE Dextran
Group (4): Vaccinated with 0.5 ml alhydragel vaccine + 25μg/ml DEAE Dextran
Group (5): Vaccinated with 0.5 ml alhydragel vaccine + 50μg/ml DEAE Dextran
Group (6): Vaccinated with 0.5 ml alhydragel vaccine + 100μg/ml DEAE Dextran
Group (7): Vaccinated with 0.5 ml oil adjuvanted vaccine only
Group (8): Vaccinated with 0.5 ml alhydragel vaccine only
Group (9): Non-vaccinated negative control group

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دراسة استخدام مادة دكستران أثناء تحضير لقاح انفلونزا الطيور الزيتى المثبط

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أجريت تجربتان فى هذه الدراسة لإضافة مادة الدكستران اثناء تحضير لقاح أنفلونزا الطيور المثبط. الأولى كان الهدف منها دراسة إضافة هذه المادة بثلاث تركيزات (25، 50، 100 ميكروجرام) لكل 0.1 ملى أثناء تخفيف الفيروس للحقن بالبيض المخصب وفى التجربة الثانية تم إضافة النسب السابقة نفسها اثناء تحضير اللقاح المثبط لكل 0.5 ملى من اللقاح التهائى.

أظهرت النتائج أن إضافة الدكستران بنسبة 25 ميكرون لكل 0.1 ملى عند حقن البيض أعطى زيادة فى القوة العيارية واعلى قراءة فى اختبار التلازن الدموى. كما أظهرت نسبة 100 ميكرون لكل 0.5 ملى عند إضافتها للقاح المثبط أثناء تحضيره أعلى عيارية من الأجسام المضادة فى الدجاج المحصن بالمقارنة بالضوابط المحصنة بدون إضافات.

ويوصى الباحث باستخدام هذه المادة لتحضير اللقاحات.