TRIALS TO IMPROVE THE IMMUNE RESPONSE OF LIVE NEWCASTLE DISEASE VACCINE

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ABSTRACT

Three experiments were designed to study the effect of addition of both DEAE dextran and casein hydrolysate in different concentrations to the live Newcastle disease (ND) vaccine each alone before lyophilization and as a constituent of the vaccinal diluent during vaccination. Also, their effect on the virus propagation in embryonated chicken eggs (ECEs) were studied. The obtained results revealed that the addition of 100µg/ml DEAE dextran to the vaccinal diluent gave the higher HI titre and 100% protection after challenge 4 weeks post vaccination followed by the group given 50 µg/ml. In case of addition of 1 and 2% casein hydrolysate before lyophilization of ND vaccine give the higher haemagglutination inhibition (HI) titre after 4 weeks post vaccination and 100% protection. The results of injection of the same concentration of both substances revealed their effect on the virus propagation in the ECEs.

INTRODUCTION

The distribution of Newcastle disease is dependent on the attempts for eradication and control made in different countries. In spite of the widespread of Newcastle disease (ND) vaccines, there are a large number of outbreaks of the disease. Outbreaks occurred in birds that had been vaccinated at least once, and in many cases the birds had received two or more live virus vaccination. The immune response is influenced by a large number of genetic and environmental factors for example heavily parasitized, malnourishment and stress factors which will inhibit the normal immune response. In the field, the failure of the birds to withstand exposure to ND may be due to inadequate amounts of virus in the vaccines. In some instances, low titre of the vaccine may be due to the mishandling. **Villasenor (1964)** found a 3.0 log drop in virus titre when the reconstituted vaccines were held at 30°C for one hour, a temperature that can be readily reached within a poultry house in tropical and subtropical countries. In the present study, two

substances (DEAE dextran and casein hydrolysate) were subjected to study of their effect on the immunogenicity of live ND vaccine.

MATERIAL AND METHODS

Viruses :

Newcastle disease vaccine :

LaSota strain (supplied by the Central Veterinary Laboratory, Weybridge, England) was used in the preparation of ND vaccine used in this study its titre was ranged from 10^9-10^{11} EID_{50}/ml.

Newcastle disease virulent virus :

Velogenic viscerotropic NDV was locally isolated and identified by **Sheble and Reda (1978)** and it was used for challenge test (I/M injection) (0.5ml/chick) containing 10^6 EID_{50}/ml and the chicks were observed for 15 days. Chicks died during this period were collected and examined for characteristic lesions of ND virus.

Embryonated Chicken Eggs (ECEs) :

9-11 days old embryonated chicken eggs obtained from United Company for Poultry Production were used in titration and safety.

Experimental birds :

Four hundred, one day old Hubbard chicks were obtained from the United Company for Poultry Production. The chicks were reared in isolated disinfected wire floor cages and fed on a complete balanced rations till 4 weeks old (where the maternal immunity weaned). The chicks were divided into 20 groups.

Casein hydrolysate (acid) :

Unipath LTD, BASINGSTOKE, HAMPSHIRE, England.

Typical analysis (90 w/w)

Total nitrogen..... 7.6

Amino nitrogen..... 4.9

Sodium chloride..... 28.3

Tryptophan..... chicken 0.1 pH (1% solution) 7.0

It was used in media for vaccine production and vaccination in concentration of 1, 2, 3% (w/v).

DEAE dextran HCl :

Diethylamine ethyl dextran M.W. 500,000 Pharmacia Fine Chemical, Sweden.

According to **Anderson et al. (1971)** a solution of it was prepared in 0.25 M Tris HCl buffer of pH 3.2. It was autoclaved and its pH adjusted to 7.6 - 7.8. It was kept at room temperature until used. It was used in concentration of 25, 50, 75, 100 and 200 ug/ml inoculum.

Newcastle disease Virus titration in embryonated chicken eggs:

It was carried out according to **Anon (1971)**. The EID_{50} was calculated by the method of **Reed and Muench (1938)**.

Haemagglutination Inhibition (HI) test :

The test was carried out according to the standard method described by **Majujable and Hitchner (1977)**.

Experimental design :

Three experiments were conducted in this study.

First experiment :

DEAE dextran were used in different concentration (25, 50, 75, 100 and 200 ug/ml) of the vaccine diluent and casein hydrolysate in concentration of 1, 2 and 3 percent) also to the vaccine diluent. Eight group of chicks (each 20 birds) were vaccinated with ND vaccine containing these concentration. Two groups were kept as negative (non vaccinated) and positive control (vaccinated with the ordinary vaccine by eye drop method).

Second experiment :

The same above mentioned concentration of both DEAE dextran and casein hydrolysate were added to the prepared vaccinal fluid (before lyophilization) then 8 groups of chicks were vaccinated with this prepared ND vaccine (2 groups were kept as positive and negative control). Challenge was carried out 4 weeks later for the 2 experiments.

Third experiment :

To estimate the effect of DEAE dextran on the ECEs, five ECEs were inoculated by 0.2ml of different DEAE dextran concentration (25, 50, 75, 100 and 200 ug/ml).

Titrations were carried out to the NDV vaccine after the addition of 25, 50, 75, 100 and 200 ug/ml DEAE dextran as well as after the addition of 1, 2 and 3% of casein hydrolysate to study the effect of both substances on the virus titre.

RESULTS AND DISCUSSION

The result of addition of the different concentration of DEAE dextran and casein hydrolysate to the vaccinal diluent (experiment 1) revealed clearly that the addition of 100µg DEAE dextran gave the higher HI titre after 4 weeks post vaccination reached (11) $\log_2$ HI titre followed by the addition of 50mg gave (9.2) $\log_2$ HI titre 4 weeks post vaccination, as well as the 2 concentration gave 100% protection. While positive control gave (3.2) $\log_2$ HI titre and 85% protection.

While the addition of casein hydrolysate in concentration 1.2, 3% gave the higher HI titre mainly 1 week post vaccination reached (8.4) $\log_2$ HI titre and 100% protection 4 weeks post vaccination.

In concern to the second experiment its results showed that the addition of DEAE dextran in concentration (25, 50 and 75µg/ml) to the vaccine before lyophilization cause a higher HI titre 1 week post vaccination reached (9.2, 8.6 and 9), respectively reached to the maximum HI titre (11) by the 3rd week post vaccination. In case of addition of (50 and 75µg/ml) DEAE dextran and 100% protection percent.

These results are in agreement to that obtained by **Mansour (1995)** and was explained by **Wittman et al. (1970)** who suggested that the adjuvant activity of DEAE dextran could be attributed to a membrane effect on the immuno-competent cells to become antibody producer. The addition of the casein hydrolysate by the 3 concentration (1, 2 and 3%) raised the HI titre from the 1st week till the 4th week post vaccination and gave 100% protection in case of addition of 2 and 3%. **Sergio and Benjamin (1971)** suggested that the buffers can containing casein hydrolysate protected NDV against thermo inactivation at 25, 37 and 45^{0}C for periods up to 60 minutes thus casein hydrolysate in the vaccine diluent appears to be desirable to protect the vaccine from adverse field conditions.

The results of experiment (3) showed that the addition of 25µg/ml DEAE dextran to the virus inoculum before inoculation of ECEs increase the virus titre (2.8) log while the addition of 200 µg/ml raised the titre by 1.5 log in comparison to the control. Similar results were obtained with **Taha (1984)** who used DEAE dextran at a concentration of 20µg/ml to increase the resulting Rift Valley Fever virus titre. The addition of the casein hydrolysate in concentration (1 and 2%) increase the virus titre by (1.6 and 1.4) log respectively in comparison to positive control.

Table 1 : Mean log_2 of Haemagglutination Inhibition (HI) titre and challenge results of the chick groups vaccinated by ND vaccine plus different concentrations of DEAE dextran in the vaccinal diluent.

Virus form	Mean log_2 HI titre				Challenge result		Protection %
	1 week	2 weeks	3 weeks	4 weeks	No. of chicks	No. of live chicks	
1	8.8	9.6	8.8	9.6	20	18	90
2	3.8	9.4	3.8	9.4	20	20	100
3	6.2	9.6	6.2	9.6	20	18	90
4	6.8	8.5	6.8	8.5	20	20	100
5	9.4	7.8	9.4	7.8	20	20	100
Cont. +ve	7.2	9.6	7.2	9.6	20	17	85
Cont. -ve	2.3	2.0	2.3	2.0	20	4	20

- * Not Done.

1st group : Vaccinated by ND vaccine with 25µg/ml DEAE dextran in its diluent.
2nd group : Vaccinated by ND vaccine with 50µg/ml DEAE dextran in its diluent.
3rd group : Vaccinated by ND vaccine with 75µg/ml DEAE dextran in its diluent.
4th group : Vaccinated by ND vaccine with 100µg/ml DEAE dextran in its diluent.
5th group : Vaccinated by ND vaccine with 200µg/ml DEAE dextran in its diluent.
Cont. +ve group : Vaccinated by ND vaccine with the normal diluent (+ve control)
Cont. -ve group : Non vaccinated control.

Table 2 : Mean log_2 of Haemagglutination Inhibition (HI) titre and challenge results of the chick groups vaccinated by ND vaccine plus different concentrations of casein hydrolysate in the vaccinal diluent.

Virus form	Mean log_2 HI titre				Challenge result		Protection %
	1 week	2 weeks	3 weeks	4 weeks	No. of chicks	No. of live chicks	
1	8.4	4.8	6.0	2.6	20	20	100
2	8.4	6.2	6.4	2.6	20	20	100
3	8.0	5.0	6.2	3.0	20	20	100
Cont. +ve	7.2	9.6	6.2	3.2	20	17	85
Cont. -ve	2.3	2.0	2.0	0.2	20	4	20

1st group : Vaccinated by ND vaccine with 1% w/v casein hydrolysate in its diluent.
2nd group : Vaccinated by ND vaccine with 2% w/v casein hydrolysate in its diluent.
3rd group : Vaccinated by ND vaccine with 3% w/v casein hydrolysate in its diluent.
Cont. +ve group : Vaccinated by ND vaccine with the normal diluent (+ve control)
Cont. -ve group : Non vaccinated control.

Table 3 : Mean log_2 of haemagglutination inhibition litre and challenge results of the groups of chickens vaccinated by ND live vaccine containing different concentrations of DEAE dextran.

Groups	Mean log_2 HI titre				Challenge results		Protection %
	1 week	2 weeks	3 weeks	4 weeks	No. of chicks	No. of live chicks	
1	9.2	6.6	7.2	5.8	20	18	90
2	8.6	8.6	11	-*	20	20	100
3	9.0	9.6	11	-	20	20	100
4	7.2	6.2	7.2	5.8	20	20	100
5	7.0	9.2	8.5	6.0	20	20	100
Cont. +ve	6.6	8.6	7.8	7.0	20	18	90
Cont. -ve	2.0	2.0	0.0	0.0	20	0	0

-* Not Done.
1st group : Vaccinated by live ND vaccine containing 25µg/ml DEAE dextran.
2nd group : Vaccinated by ND vaccine containing 50µg/ml DEAE dextran.
3rd group : Vaccinated by ND vaccine containing 75µg/ml DEAE dextran.
4th group : Vaccinated by ND vaccine containing 100µg/ml DEAE dextran.
5th group : Vaccinated by ND vaccine containing 200µg/ml DEAE dextran.
Cont. +ve group : Vaccinated by ND usual live ND vaccine.
Cont. -ve group : Non vaccinated control.

Table 4 : Mean log2 of haemagglutination inhibition (HI) titre and challenge results of different groups of chickens vaccinated by ND live vaccine containing different concentrations of casein hydrolysate.

Virus form	Mean log_2 HI titre				Challenge results		Protection %
	1 week	2 weeks	3 weeks	4 weeks	No. of chicks	No. of live chicks	
1	8.2	6.8	6.4	4.4	20	17	85
2	8.6	9.0	8.8	9.0	20	20	100
3	8.2	8.2	8.2	8.6	20	20	100
Cont. +ve	6.6	8.6	7.8	7.0	20	18	90
Cont. -ve	2.0	2.0	0.0	0.0	20	0	0

1st group : Vaccinated by ND vaccine containing 1% casein hydrolysate.
2nd group : Vaccinated by ND vaccine containing 2% casein hydrolysate.
3rd group : Vaccinated by ND vaccine containing 3% casein hydrolysate.
Cont. +ve group : Vaccinated by usual live ND vaccine.
Cont. -ve group : Non vaccinated control.

Table 5 : Titration results after addition of DEAE dextran in different concentration to the Newcastle disease virus inoculum.

Concentration (µg/ml)	Titre EID_{50}
25	11.8
50	9.3
75	0.0
100	9.1
200	10.5
Control	9.0

Table 6 : Titration results after addition of casein hydrolysate in different concentration to the Newcastle disease virus inoculum.

Concentration (%)	Titre EID_{50}
1	12.4
2	12.6
3	8.4
Control	11.0

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الملخص العربى

محاولات لتحسين القوة المناعية للقاح النيوكاسل الحى

المشتركون فى البحث

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فى محاولة لتحسين الكفاءة المناعية للقاح النيوكاسل الحى تم إضافة مادتين هما الكازيين والديكستران إلى لقاح النيوكاسل مرة قبل التجفيف وأخرى فى مذيب اللقاح أثناء التحصين فى تجربتين منفصلتين. ففى التجربة الأولى تم إضافة مادة الديكستران بنسب ٢٥، ٥٠، ٧٥، ١٠٠، ٢٠٠ ميكرون لكل سم٣ لقاح ومادة الكازيين بنسبة ١، ٢، ٣٪ على مذيب اللقاح أثناء التحصين. وفى التجربة الثانية تم إضافة نفس النسب ولكن على اللقاح أثناء التحضير وقبل التجفيف. ولكل من التجربتين تم تحصين مجموعات من الكتاكيت عمر ٤ أسابيع وتم قياس الأجسام المناعية باختبار (HI) إسبوعياً وتم إجراء إختبار التحدى عند أربعة أسابيع.

وقد وجد أن المجموعة التى حصنت بلقاح النيوكاسل الحى عترة (لاسوتا) إضافة إلى الديكستران بنسبة ١٠٠ ميكرون لكل سم٣ مذيب قد أعطت أعلى مستوى من الأجسام المناعية فى الإسبوع الرابع بلغ متوسط لوغاريتم ١١ تلتها المجموعة التى أضيف لها ٥٠ ميكرون لكل سم٣ مذيب وبلغت متوسط لوغاريتم ٩ر٢ وبلغت نسبة الحماية لكل منهما ١٠٠٪. بينما بلغت المجموعتان التى أضيف لها الكازيين أثناء تحضير اللقاح بنسب ١، ٢٪ أعلى متوسط لوغاريتم عند الإسبوع الرابع وكان ٩، ٦، ٨ على التوالى بفارق ٢ لوغاريتم عن اللقاح العادى. كذلك تم دراسة تأثير تلك المادتين على عيارية الفيروس فى البيض المخصب وقد وجدت أنها ارتفعت بمقدار ٨ر٢ لوغاريتم فى حالة إضافة ٢٥ ميكرون من الديكستران أثناء حقن البيض و ٥ر١ لوغارتيم عند إضافة ٢٠٠ ميكرون مقارنة بالمجموعة الضابطة . كما زادت عيارية فيروس النيوكاسل بنسبة ٦ر١-٤ر١ عند إضافة الكازيين بنسب ١، ٢٪ على التوالى عند حقن البيض.