PRELIMINARY TRIALS FOR PREPARATION OF A BIVALENT INACTIVATED VACCINE AGAINST NEWCASTLE DISEASE AND EGG DROP SYNDROME

By

Abo-Zaid A. Abo-Zaid; Khodeir, M.H.; Nadia M. Hassan; Fekria El-Bourdini and Mohab H. Awad

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

ABSTRACT

Inactivated oil monovalent Newcastle disease (ND) and egg drop syndrome (EDS) vaccines as well as an inactivated bivalent (ND and EDS) vaccine were prepared in the present study and evaluated according to the directions of the recommended protocols of poultry vaccines. Such vaccines were found to be free from foreign contaminants, safe and immunogenic for chicken. The protective activity of the bivalent vaccine was compared to that of monovalent vaccines in different groups of chicken vaccinated against ND and EDS using monovalent and bivalent vaccines and compared with the imported bivalent vaccine (ND and EDS). Serologically, it was found that all vaccinated chickens had protective levels of humoral antibodies against ND and EDS either with the use of monovalent or bivalent vaccines without showing any antagonizing effect between the immune response of chicken to ND and EDS.

INTRODUCTION

Poultry industry plays a very important role providing human being with animal protein which is an essential component for good health especially in growing children. In addition, poultry industry shares with a good part in the national income. However, this industry faces many great problems and one of them could implicate it greatly as the outbreaks of viral diseases. Newcastle disease (ND) and Egg drop syndrome (EDS) are two viral diseases which cause great economic losses either through the high mortality rate (ND), loss of weight, low egg production or deformed eggs (**Van Eck *et al.*, 1976 and Holmes *et al.*, 1989**).

Vaccination of chicken against such viral diseases has been succeeded either by using of monovalent or bivalent vaccines without an antagonizing effect of the including antigens (**Allan *et al.*, 1973; Baxendal *et al.*, 1980;**

Mouaz, 1986; Madkour, 1995; Hala, 1996; Holmes *et al.*, 1989; khodeir and Amina, 1999 and Afaf *et al.*, 2000).

The present work was designed to prepare an inactivated bivalent vaccine against both of ND and EDS in a trial to safe time, effort and stress factors on the birds and compare it with the imported bivalent vaccine.

MATERIAL AND METHODS

1. Viruses:

1.1. Newcastle disease virus (NDV):

NDV (LaSota strain) was supplied by the Central Veterinary Laboratory, Weybridge, England. It had a titre of 10^{11} EID_{50}/ml.

1.2. Egg drop syndrome (EDS) virus:

EDS-76, Code PA0081 was kindly supplied by Weybridge, England.

1.3. Imported inactivated bivalent vaccine:

Vaccine against Egg drop syndrome-76 and Newcastle disease in chickens, Intervet International B.V. Boxmeer, Holland.

2. Embryonated eggs:

Commercial embryonated duck eggs and embryonated chicken eggs were obtained from United Company for Poultry Production. The embryonated duck eggs were used for propagation of the virus, testing of complete virus inactivation and preparation of batch of EDS inactivated vaccine. The embryonated chicken eggs were used for preparation of NDV vaccine.

3. Cell cultures:

African green monkey kidney cells (VERO) established by **Yasumura and Kawatika, (1963)** were used in SNT to estimate ND neutralizing antibodies in the sera of vaccinated chickens.

Chicken embryo rough cells (CER) established by **Smith *et al.*, (1977)** were used for titration of EDS neutralizing antibodies in the sera of vaccinated chickens using SNT.

4. Experimental chicks:

Commercial one hundred and fifty, one day old Hubbard chicks were supplied by the United Company for Poultry Production. The chicks were reared under complete hygienic measures in isolated and disinfected wire floored cages and fed commercial broiler ration.

5. Virus propagation:

5.1. NDV was propagated in embryonated chicken eggs according to **Allan *et al.*, (1973)**. The obtained virus was titrated according to the standard methods described in **Reed and Muench, (1938)**. It had a titre of 10^{11} EID_{50}/ml.

5.2. EDSV was propagated in embryonated duck eggs according to **Allan *et al.*, (1973)**. The obtained virus was titrated in embryonated duck eggs and the titre was expressed and calculated according to the method of **Reed and Muench, (1938)** and was found to be 10^7 EID_{50}/ml.

6. Inactivation of viruses:

Inactivation of the used viruses were carried out using formalin in a final concentration of 0.1% of the total volume for both NDV and EDS. The fluid was left on a magnetic stirrer for 18 hours for ND (**Mohab, 1992**) and for 48 hours to EDS (**Rozhdesvenskii, 1984**) at room temperature.

Judgement for inactivation was carried out by inoculating the inactivated virus samples in embryonated chicken eggs (0.2ml/egg) for NDV while EDS was tested in embryonated duck eggs to be sure that a complete inactivation occurred.

7. Vaccine preparation:

The monovalent oil vaccines against each virus as well as the bivalent inactivated oil vaccine were prepared according to the method of **Stone *et al.*, (1978)** with aqueous to oil ratio 1:3. The prepared vaccines were adjusted to contain 10^9 EID_{50} of ND and 10^7 EID_{50} for EDS / dose.

8. Experimental design:

One hundred and fifty, one-day-old chicks were reared till they became 4 months old. The chicks were divided into 5 groups (30 chicks/each group).

Group (1): Vaccinated with the prepared bivalent inactivated oil vaccine.

Group (2): Vaccinated with the imported inactivated oil vaccine.

Group (3): Vaccinated with the locally prepared inactivated monovalent oil ND vaccine.

Group (4): Vaccinated with the locally prepared inactivated monovalent oil EDS vaccine.

Group (5): Non-vaccinated controls.

Each chick of vaccinated groups received 0.5 ml S/C from the prepared vaccines according to its group.

Ten random blood samples were collected weekly from each group for 24 weeks post vaccination. Sera were collected and stored at -20°C until used for detection of corresponding antibodies against ND and EDS.

9. Haemagglutination Inhibition test (HI):

It was used for estimating the haemagglutinating inhibiting antibodies against ND and EDS viruses. It was done according to **Majujabe and Hirchner, (1977)**.

10. Serum neutralization test (SNT):

It was used for estimating the neutralizing antibodies against both ND and EDS. The test was carried out after the method of **Weisman and Hitchner, (1978).**

RESULTS AND DISCUSSION

It is well known that poultry meat is one of the main sources of animal protein, which is an essential component in healthy food for building human body. Poultry industry plays an important role in solving the problem of the decrease in beef production especially in developing countries where there is an increased population or low income. So, the improvement of poultry industry and its related services, is a main goal to veterinarians and commercialists.

One of the most essential parameters in poultry industry is the protection of poultry against the infectious diseases which cause a dramatic losses. Such protection depends mainly on the successive vaccination with specific immunogenic vaccines. Nowadays, the attention of workers, directed towards the use of combined vaccines in trials to decrease the stress factors on birds; due to different vaccinations on different periods; and to safe time and cost.

In the present study, an inactivated combined vaccine was prepared against two of poultry infectious diseases, which affect greatly on poultry production; EDS and ND. The protective activity of such vaccine was compared with that of an imported one and with that of monovalent vaccines against EDS and ND.

Table (1) showed that in all vaccinated chicken groups exhibited specific EDS neutralizing antibodies from the 1st week post vaccination. These antibodies increased gradually to reach their peak (85.33) in all groups without any antagonizing effect between EDS and ND on the immune response of vaccinated chickens. Also, the results of HI test (Table 2) showed good levels of immunity in all groups and confirmed the results of SNT. In addition, the egg production of vaccinated birds did not be affected (neither the quality nor the quantity). These results agree with **Nedelciu and Sofei, (1990) and Uslangolu, (1990)** who reported that EDS vaccine in a combination with ND and IBD did not affect egg production and induced good levels of immunity up to 24 weeks post vaccination as obtained in the present study. Also, **Khodeir and Amina, (1999) and Bidin *et al.*, (1998)** recorded similar results.

On the other side of view, Tables (3 and 4) revealed the results of SNT and HI test which declared that the vaccinated chickens in all groups exhibited high levels of specific ND antibodies up to 24 weeks post vaccination (the experimental period). These antibodies were detectable from the 1st week post vaccination without an undesirable effect of the combination between EDS and ND vaccine. These findings come in a complete agreement with those of **Nedelciu and Sofei, (1990); Uslangolu, (1990); Bidin *et al.*, (1998) and Holmes *et al.*, (1989)** who obtained high antibody titres in vaccinated chickens

vaccinated with combined vaccines against EDS, ND and IBD without significant difference in the levels of induced antibodies. Also, **Madkour, (1995); Hala, (1996) and Afaf *et al.*, (2000)** showed that ND did not affect the immune response of chicken against other antigens in case of its combination with them in combined vaccines.

It is of important to mention that there was no detectable difference between the immunogenicity of locally prepared and imported vaccines showing that the local vaccine is a good vaccine. Also, the quality control test of the prepared vaccines showed that they are safe, immunogenic and free from foreign contaminants according to the directions of standard protocols **(Davidson, 1975 and Anon, 1994)**.

It could be concluded that the usage of prepared combined EDS and ND inactivated vaccine protects chicken against both diseases at the same time.

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Table (1): Neutralizing EDS antibody titres in vaccinated chickens

Used vaccine	Mean neutralizing EDS antibody titres* / Weeks Post Vaccination							
	1	2	3	4	8	12	20	24
Locally prepared bivalent EDS & ND vaccine	2	5.33	16	53.33	64.0	85.33	53.33	32.0
Imported bivalent EDS & ND vaccine	2	6.33	26.66	85.33	85.33	74.66	26.66	42.66
Monovalent local EDS vaccine	3.33	6.33	32	64	85.33	74.66	48.0	21.33
Control group	0	0	0	0	0	0	0	0

* Antibody titre = reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of EDS virus.

Table (2): EDS HI antibody titres in vaccinated chickens

Used vaccine	Mean HI titre of EDS (log_2) / Weeks Post Vaccination							
	1	2	3	4	8	12	20	24
Locally prepared bivalent EDS & ND vaccine	2	4	16	64	64	128	256	64
Imported bivalent EDS & ND vaccine	2	4	32	64	64	128	64	64
Monovalent local EDS vaccine	0	8	16	64	64	128	128	64
Control group	0	0	0	0	0	0	0	0

Table (3): ND neutralizing antibody titres in vaccinated chickens

Used vaccine	Mean ND neutraliziug antibody titres* / Weeks Post Vaccination							
	1	2	3	4	8	12	20	24
Locally prepared bivalent EDS & ND vaccine	2	6.66	13.33	42.66	106.66	128	128	128
Imported bivalent EDS & ND vaccine	2	5.33	16	53.33	96.0	128	128	128
Monovalent local ND vaccine	4	8	16	64	128	128	128	128
Control group	0	0	0	0	0	0	0	0

* Antibody titre = reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of ND virus.

Table (4): ND HI antibody titres in vaccinated chickens

Used vaccine	Mean ND HI titre (log_2) / Weeks Post Vaccination							
	1	2	3	4	8	12	20	24
Locally prepared bivalent EDS & ND vaccine	2	8	8	64	128	256	256	128
Imported bivalent EDS & ND vaccine	2	8	16	64	256	256	256	128
Monovalent local ND vaccine	4	8	16	32	256	256	256	256
Control group	0	0	0	0	0	0	0	0

Fig. (1): Neutralizing EDS antibody titres in vaccinated chicks

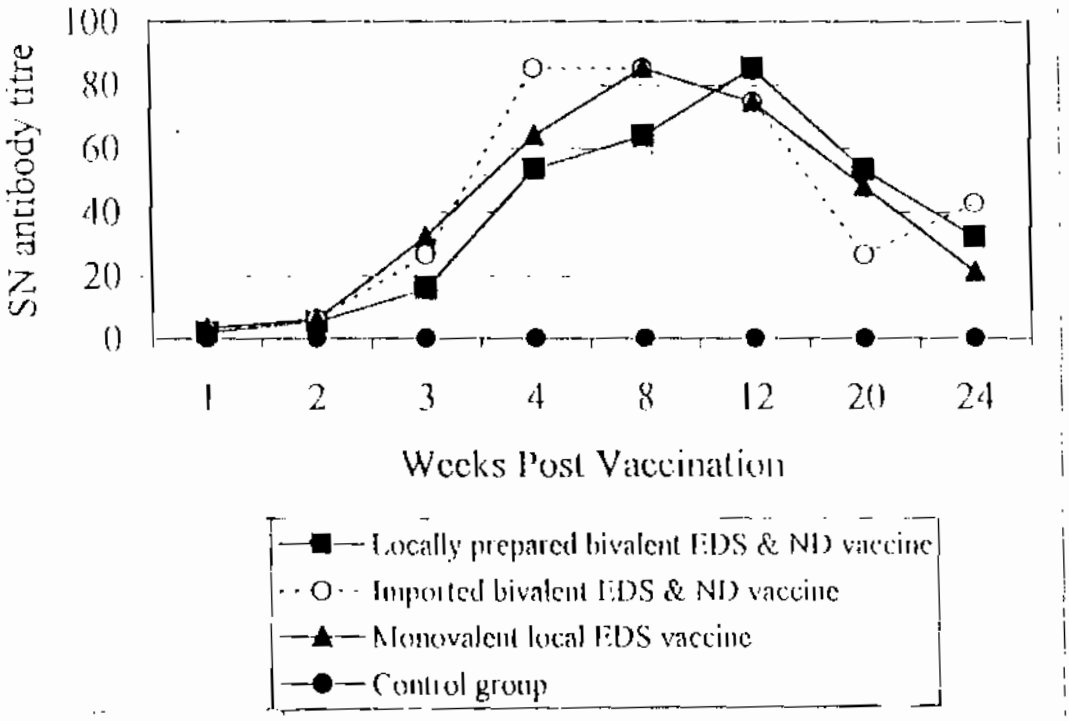


Fig. (2): EDS HI antibody titres in vaccinated chicks

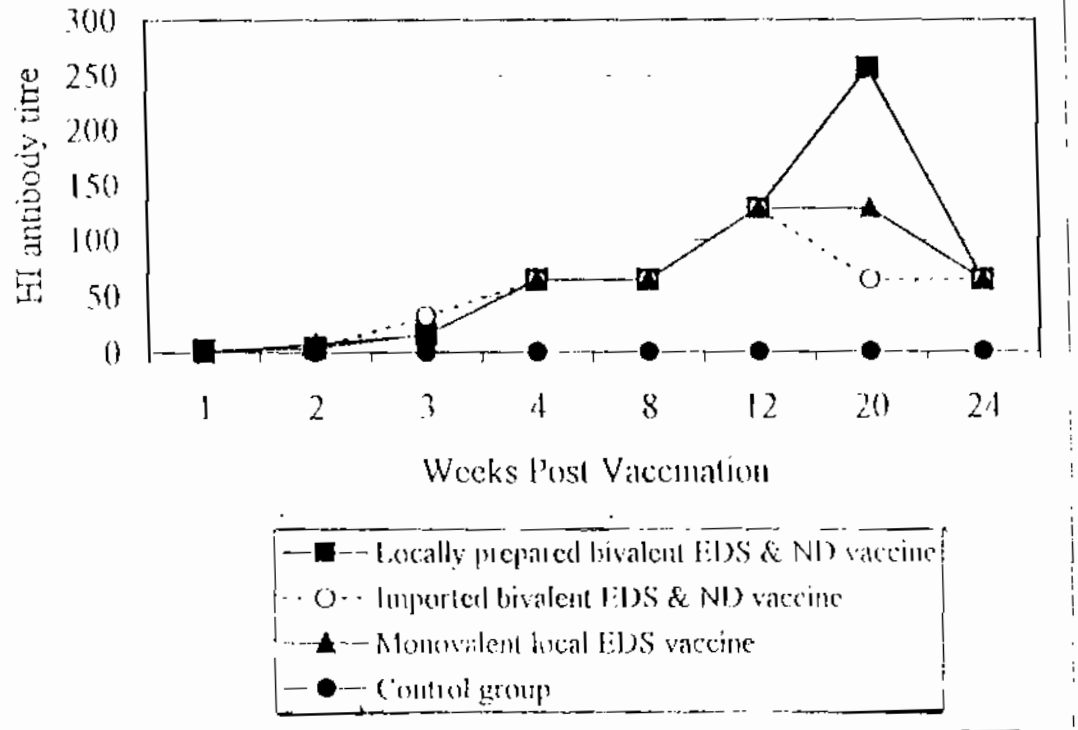


Fig. (3): Neutralizing ND antibody titres in vaccinated chicks

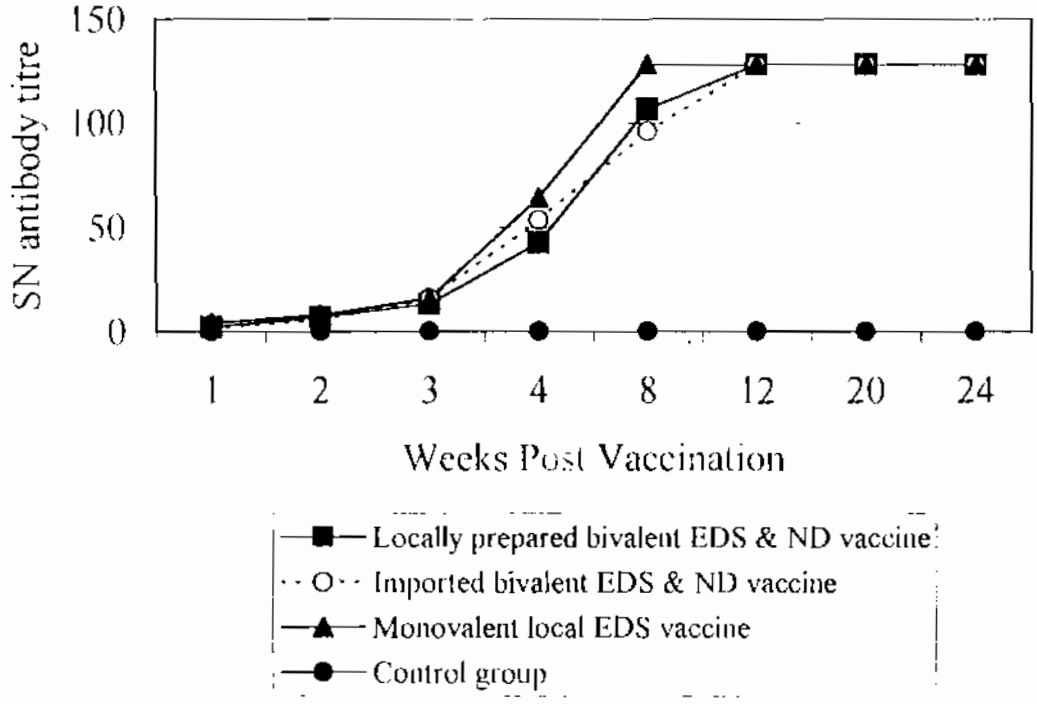
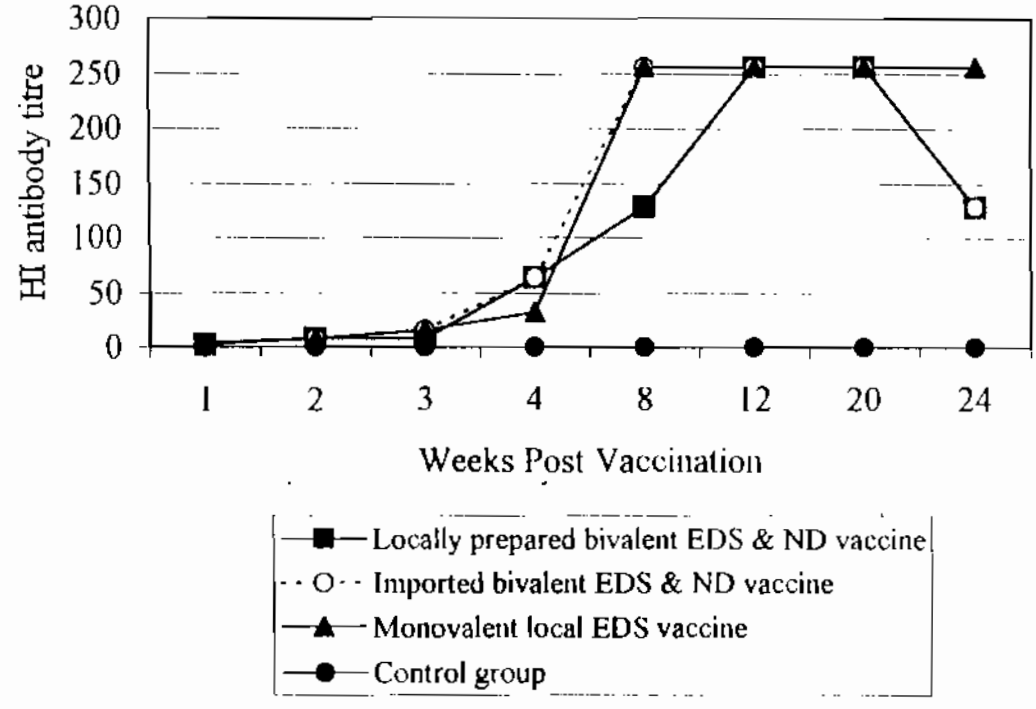


Fig. (4): ND HI antibody titres in vaccinated chicks



الملخص العربي

محاولات مبدئية لتحضير لقاح ثنائي ميت ضد مرض النيوكاسل وظاهرة انخفاض البيض

أبو زيد أحمد أبو زيد محمد حسن خضير نادية محمد حسن

فكرية عبد الحافظ البردينى مهاب حليم عوض

معهد بحوث الأمصال واللقاحات البيطرية – العباسية – القاهرة

تم تحضير لقاح ثنائي زيتي ميت ضد مرض النيوكاسل وظاهرة تدنى البيض بالإضافة إلى تحضير لقاح أحادى ضد كل فيروس على حدة. تمت مقارنة فاعلية اللقاح الثنائي المحضر محلياً ومثيله المستورد وذلك بحقن كل لقاح في مجموعات من الدجاج وتم جمع عينات المصل لمدة ٢٤ أسبوع بعد التحصين وقد أوضحت النتائج السيرولوجية أن اللقاح الزيتي الميت المحضر محلياً يعادل اللقاح المستورد في الكفاءة المناعية حيث استمرت هذه الكفاءة المناعية عالية حتى الشهر السادس بعد التحصين بكل لقاح وأن اللقاح الأحادي يماثل اللقاح المزدوج في الكفاءة المناعية مما يوضح أن اللقاحات المركبة لا يؤثر بعضها البعض على الاستجابة المناعية للطيور بالإضافة إلى أنها تغنى عن استخدام عدة لقاحات في تحصين الدجاج وتوفر بذلك الوقت والجهد والمال وهى فعالة وآمنة تقي الطيور ضد كلا المرضين.