PRELIMINARY TRIALS FOR PRODUCTION OF TRIVALENT INACTIVATED OIL VACCINE AGAINST NEWCASTLE, INFECTIOUS BRONCHITIS AND EGG DROP SYNDROME DISEASES

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ABSTRACT

Newcastle disease virus (NDV), egg drop syndrome (EDS) and infectious bronchitis (IBV) combined trivalent and monovalent oil adjuvant vaccines were prepared and tested for safety and immunogenicity in 4 week-old commercial chickens. The chickens vaccinated with a dose of 0.5ml developed satisfactory levels of antibodies to ND, EDS and IB viruses. The results showed that no significant differences in antibody titres between the respective groups up to 8 week observation period. So, the trivalent vaccine was safe and immunogenic against NDV, EDSV and IBV in one dose.

INTRODUCTION

Viruses that infect the respiratory organs of poultry continue to cause serious disease problems throughout the world, although often the most serious consequences of infection are the result of invading other organs or tissues after infection is established in the respiratory tract, or exacerbation in dual or multiple infections with other organisms. Newcastle disease (ND) is a serious disease causing high mortality, and low productive performance **(Biswal and Morril, 1954).** Similarly, infectious bronchitis virus (IBV) is primarily respiratory disease, together with egg production problems that may occur when infection of oviduct are of early ages. Significant mortality may occur in young ages particularly if the disease is exacerbated by secondary pathogens **(Hofstad, 1984)**. Egg drop syndrome (EDS) causes severe damage in the uterus with production of soft shelled cracked eggs of poor albumin quality **(Swain et al., 1993)**.

The combined vaccines have the advantage of providing protection against more than one disease at the same time thus reducing vaccination expensive and number of vaccination per farm as well as saving time and labour costs. Besides that, combined vaccines reduces the stress re-

actions. So, the aim of this work is to produce trivalent vaccine to protect the chickens from these serious diseases in one dose.

MATERIAL AND METHODS

Viruses :

1. Newcastle disease virus :

LaSota strain (supplied by the Central Veterinary Laboratory, Weybridge, England).

2. Infectious bronchitis disease virus :

Serum H120 was obtained as allantoic fluid from Department of Animal Science and Agricultural Biochemistry, University of Delmare, New York, USA.

3. Egg drop syndrome disease virus :

EDS-76 live antigen product code PA0081 was handled by **Prof. Dr. Nadia M. Hassan**, from Weybridge, England.

Embryos :

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

Cell cultures :

African green monkey kidney cells (Vero) established by **Yasumara and Kawatika (1963)** were used in SNT to estimate IB neutralizing antibodies in the sera of vaccinated chickens.

Chicken embryo rough cells (CER) established by **Smith et al. (1977)** were used for detection of EDS neutralizing antibody titres in sera of vaccinated chickens using SNT.

Experimental chicks:

One hundred and fifty, one day old mixed sex commercial 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.

Virus Propagation:

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 **FAO Publication (1978)**. It had a titre of 1011 EID50/ml.

2. IBV was propagated and titrated according to method described by **Cuningham (1973)**. The titre of the virus was 109 EID50/ml.
3. EDSV was propagated according to **Allan et al. (1973)** and titrated in embryonated duck eggs. The titre was expressed and calculated according to the method of **Reed and Muench (1938)** and found to be 107 EID50/ml.

Inactivation of viruses :

Inactivation of the used viruses were separately carried out using formalin in a final concentration of 0.1%. The fluids were left on a magnetic stirrer at room temperature for 18 hours for ND and IB and for 48 hours for EDS.

Judgement for inactivation was carried out by inoculation of samples from treated viruses in embryonated chicken eggs (0.2 ml/egg) for NDV and IB, while EDS was tested in embryonated duck eggs to be sure that the required complete inactivation occurred.

Vaccine Preparation:

The monovalent oil vaccines against each virus as well as the trivalent inactivated oil vaccine were prepared according to **Stone et al. (1978)** with aqueous to oil ratio 1:3. The prepared vaccines were adjusted to contain 109, 108.5 and 107 for ND, IB and EDS / dose, respectively.

Quality Control :

The prepared vaccines were subjected for quality control measures as described by **Code of Federal Regulations, USA (1987)**.

Serological tests:

1. Haemagglutination Inhibition (HI) test :

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

2. Serum neutralization test (SNT):

It was used for estimating the neutralizing antibodies against both IB and EDS after methods of **Rossiter et al. (1985)**.

3. ELISA test:

The IDEXX ELISA kits (flock check system) were used to determine the level of serum antibodies against IBV.

Experimental Design :

One hundred and fifty, one day old chicks were reared in an isolated conditions. The chicks were divided into 5 groups (30 chicks / each group):

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

Group (2): Vaccinated with locally prepared inactivated monovalent oil ND vaccine.

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

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

Group (5): Non-vaccinated controls.

Each chicken in the vaccinated groups received 0.5 ml I/M injection from the prepared vaccines according to its group at 30 days of age. Ten random blood samples were collected weekly from each group for 8 weeks post vaccination. Sera were collected separately and stored at -20°C until used for detection of corresponding antibodies against ND, IB and EDS.

RESULTS AND DISCUSSION

Results obtained shown in Table (1) revealed that the HI antibody response against NDV in group (1) (trivalent vaccine) and group (2) (monovalent vaccine of NDV) increasing of HI antibody titre gradually from first week until the 8th week in the two groups reaching maximum titre and there was no difference between the two groups (trivalent and monovalent vaccines). On the other hand, in Table (2) the neutralizing antibody titres in group (1) (vaccinated with trivalent vaccine) and group (3) (vaccinated with monovalent IB vaccine) showed that the serum neutralizing antibody titre increased from the first week reached maximum titre in 8th week in two groups and recorded that the monovalent vaccine of IBV showed high titre in the first five weeks than the trivalent vaccine and became at the same level in the next weeks. These results agreed with that of **Kozlina et al. (1990)** who mentioned that there were no significant differences in antibody titres between the groups received trivalent vaccine or monovalent vaccine. Also, **Nedelciu and Sofei (1990)** who mentioned that groups of chickens were inoculated with oil inactivated vaccines, either bivalent (ND and IB) or trivalent (ND, EDS and IB) showed higher immunogenicity than with single vaccines. ELISA antibody titres against IBV showed in Table (3) ensured the above results of groups which were vaccinated by trivalent vaccine group (1) or monovalent IB vaccine group (3).

Regarding the results of EDS in Table (4) showed that the HI antibody titre of group (1) triva-

lent vaccine and monovalent vaccine of EDS very low in the first 3 week and then increased from 4th week till the 8th week in the two groups. Also, in Table (5), the neutralizing antibody titres appeared low at the first 3 weeks and increased gradually from the 4th week reaching maximum titre at the 8th week. So, these results of HI and SNT against EDS indicated that there is no significant difference between groups vaccinated by trivalent vaccine or monovalent vaccine. These results agreed with those obtained by **Wu-Yan Gong et al. (1994)**.

Regarded to results obtained by **Madkour et al. (1999)** who recorded that trivalent vaccine against ND, IB and IBDV was more potent than the monovalent vaccine. Also, the results agreed with those obtained by **Madkour et al. (1998)**.

So that, the present investigation indicated that the prepared trivalent oil vaccine could elicit the production of protective antibody titres against the three used viruses. No mutual enhancement or competition was detected. Similar observations were recorded by **Thayer et al. (1983)** where they recorded that no practical difference in amplitude of antibody response when ND antigen used alone or combined with IB antigen. The obtained results were also confirmed with those of **Kolchi and Yoshikazu (1973), Gough et al. (1977), Gaafar (1996) and El-Mahdy et al. (1999)** where they found that a satisfactory immune response to IB antigen could be obtained when evaluated under laboratory condition in a combined inactivated vaccine with ND virus without any antagonistic action from each other. In conclusion, the locally prepared trivalent oil inactivated vaccine was found more potent, efficient and protect the birds against the three diseases in one dose.

Table 1 : NDV HI geometric mean titres in vaccinated chickens.

Chicken groups	ND HI antibody titres (log2) / weeks post vaccination							
	1	2	3	4	5	6	7	8
1	2	16	32	128	128	256	256	256
2	0	8	16	64	128	256	256	256
5	0	0	0	0	0	0	0	0

Group (1) : Vaccinated with locally prepared trivalent vaccine.
Group (2) : Vaccinated with locally prepared monovalent NDV.
Group (5) : Control non-vaccinated.

Table 2 : IBV neutralizing antibody titres in vaccinated chickens.

Chicken groups	IBV SN antibody titres / weeks post vaccination							
	1	2	3	4	5	6	7	8
1	2	8	32	64	64	128	256	256
3	4	16	64	128	128	128	256	256
5	0	0	0	0	0	0	0	0

Group (1) : Vaccinated with locally prepared trivalent vaccine.
Group (2) : Vaccinated with locally prepared monovalent NDV.
Group (5) : Control non-vaccinated.

Table 3 : Geometric mean ELISA antibody titre against IBV in vaccinated chickens.

Chicken groups	ELISA antibody titres / weeks post vaccination							
	1	2	3	4	5	6	7	8
1	994	1988	1260	1587	3313	3910	5550	4497
3	856	2673	2277	2120	3153	3898	3931	5420
5	0.089	0.089	0.089	0.074	0.089	0.074	0.074	0.084

Group (1): Vaccinated with locally prepared trivalent vaccine.
Group (3): Vaccinated with locally prepared monovalent IBV.
Group (5): Control non-vaccinated.
N.B. Absorbance value of negative control = 0.074-0.089.
Absorbance value of positive control = 0.411-0.409.

Table 4 : EDS HI antibody titres in vaccinated chickens.

Chicken groups	EDS HI antibody titres (log_2) / weeks post vaccination							
	1	2	3	4	5	6	7	8
1	0	2	2	16	64	128	128	256
4	0	4	8	16	128	128	256	256
5	0	0	0	0	0	0	0	0

Group (1) : Vaccinated with locally prepared trivalent vaccine.
Group (4) : Vaccinated with locally prepared monovalent EDS.
Group (5) : Control non-vaccinated.

Table 5 : EDS neutralizing antibody titres in vaccinated chickens.

Chicken groups	IBV SN antibody titres / weeks post vaccination							
	1	2	3	4	5	6	7	8
1	2	2	4	8	64	128	128	128
4	0	4	4	16	64	128	128	128
5	0	0	0	0	0	0	0	0

Group (1) : Vaccinated with locally prepared trivalent vaccine.
Group (2) : Vaccinated with locally prepared monovalent EDS.
Group (5) : Control non-vaccinated.

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الملخص العربى

محاولات مبدئية لتحضير لقاح ثلاثى مثبط زيتى ضد أمراض النيوكاسل والالتهاب الشعبى المعدى وتدنى البيض فى الدجاج

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تم تحضير لقاح زيتى مثبط ضد أمراض النيوكاسل وظاهرة تدنى البيض والالتهاب الشعبى المعدى وتم اختبار كفاءة اللقاح بعدة إختبارات سيرولوچية وذلك بتحصين مجموعات مختلفة من الدجاج باللقاح الزيتى الفردى لكل مرض على حدة، وقد ثبت أن اللقاح الزيتى الثلاثى المحضر محلياً ذو كفاءة مناعية عالية وآمن لتحصين الطيور، وهذا اللقاح فعال فى وقاية الدجاج ضد الأمراض الثلاثة وهو يوفر الوقت والجهد والتكاليف التى تبذل فى التحصين بلقاح فردى.