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PREPARATION AND FIELD APPLICATION OF INACTIVATED TRIVALENT VACCINE AGAINST NEWCASTLE, INFECTIOUS BRONCHITIS AND EGG DROP SYNDROME DISEASES

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SUMMARY

A trivalent vaccine against Newcastle disease virus (NDV), egg drop syndrome virus (EDS) and infectious bronchitis virus (IBV) were prepared and tested for potency, safety and sterility. Ten thousands, 120 day old broiler breeder chickens were divided into two equal groups; the first group was vaccinated by the trivalent prepared vaccine and compared by the second group which was vaccinated by each of monovalent ND, IB and EDS oil emulsion vaccines. The results of the trivalent vaccine showed no noticeable differences in immune response as well as egg production in both groups. The trivalent vaccine saves the costs and effort than the vaccination with monovalent vaccines.

INTRODUCTION

Newcastle disease virus (NDV) causes great economic losses due to high rates of mortality, reduction of meat and drop in egg production (Biswal and Morril, 1954).

Inactivated vaccines could induce satisfactory immunity comparable to live ones (Box and Furminger, 1975).

Infectious bronchitis (IB) is a highly contagious respiratory disease of chickens caused by infectious bronchitis virus, characterized by high morbidity with low mortality rates and drop in egg production and egg quality (Hofstad, 1997). Egg drop syndrome is considered as major cause of loss of egg production through the world and sever damage in the uterus (Swain et al., 1993). An inactivated vaccine is effective in the control of

EDS-76 (Baxendale et al., 1980). The combined inactivated oil emulsion vaccine have the advantage of providing protection against more than one disease at the same time and giving high levels of humoral antibody which provide effective protection against field challenge viral infections (Thayer et al., 1983).

The combined vaccines reduce vaccination expensive and number of vaccination per farm as well as saving time and labour costs. Besides that, combined vaccines reduce the stress reaction. So, the aim of this work was to produce trivalent vaccine to protect the chickens from these serious diseases in one dose and evaluate the immune response of this vaccine in a field broiler flock.

MATERIAL AND METHODS

1. Viruses:

a. Newcastle disease virus:

LaSota strain with EID_{50} 10^{11}/ml SPF egg adapted vaccinal strain supplied by the Central Veterinary Laboratory Weybridge, England. Propagated in SPF eggs according to Allan et al. (1973).

b. Infectious bronchitis disease virus:

H120 strain was obtained as allantoic fluid from University of Delmare, New York, USA has 10^8/ml titre according to Cunningham (1973).

c. Egg drop syndrome disease virus:

EDS-76 virus strain was supplied by the Central Veterinary Laboratory, Weybridge, England with 10^8/ml titre.

2. Embryos:

a. Commercial embryonated 9-10 days old duck eggs was obtained from United Company from Poultry Production and used for EDS virus propagation according to Allan et al. (1973) and was titrated according to Reed and Muench (1938).

b. 9-10 day old embryonated chicken specific free (SPF) eggs were obtained from Ministry of Agriculture, Koum Oshiem, Fayoum, Egypt and used for IB and NDV virus propagation and titration.

3. Chickens:

Ten thousands, 120 day old broiler chickens were used for field application of the trivalent inactivated oil emulsion prepared vaccine.

4. Inactivated trivalent vaccine preparation:

The monovalent oil emulsion vaccines against each virus as well as the trivalent inactivated oil emulsion vaccine were prepared according to Stone et al. (1978) and Madkour et al. (2001) with aqueous to oil ratio 1:3 and using formalin inactivator in final concentration 1% for NDV, IBV and EDS viruses inactivation.

5. Serological tests:

Haemagglutination Inhibition (HI) test:

It was used for estimating the HI antibodies against ND and EDS viruses according to Maju-

jabe and Hitchner (1977) in serum samples of vaccinated chickens.

Serum neutralization test (SNT):

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

ELISA test:

The ELISA kits were used to determine the level of serum antibodies against IBV from (KPL Laboratories, Maryland, USA) according to the manufacturer's instructions.

5. Experimental Design:

A total number of 10,000, 120 day old commercial broiler chicks were divided into two groups each of 5000.

The first group was vaccinated by the trivalent ND, IB and EDS inactivated oil emulsion prepared vaccine at 120 day old with 0.5ml/bird S/C.

The second group was vaccinated at 120 day old by ND, IB and EDS inactivated individual monovalent oil vaccine.

Random serum samples (10%) were tested serologically for nine successive months.

RESULTS AND DISCUSSION

Newcastle disease, Infectious Bronchitis and Egg Drop Syndrome (ND, IB and EDS) are highly infectious viral diseases in poultry causing sever losses in farms with high mortality, low egg production quantity and poor egg quality.

Vaccination is the best way for viral diseases protection (Biswal and Morril, 1954 and Saif et al., 2003).

Great attention is directed toward poultry combined inactivated vaccines to save time, labour, costs, and reduce stress on chicken by many numbers of monovalent vaccinations (Stone et al., 1978).

Data in table (1) showed high neutralizing antibody titre against IBV which reached its peak on the 4th month after vaccination by both monovalent and trivalent prepared vaccine with no difference between them during the whole experimental period. Similar results were reported by Kolchi and Yashikazu (1973), Lamiaa (1996) and Nancy (2001) who found that satisfactory immune response could be obtained when evaluated under laboratory condition in a combined inactivated IB and ND vaccines without any antagonistic action from each other.

The results of ELISA antibody titre of chickens vaccinated with trivalent or monovalent IB vaccine in table (2) showed that there was a neglicable differences between both groups of chickens. This agreed with Gough et al. (1977) and Lamiaa (1996) who found no noticeable differences in

antibody titre in chickens vaccinated with trivalent ND, IB and infectious coryza and monovalent IB inactivated oil emulsion vaccine.

Results obtained in tables (3, 4) revealed an increase in SN titre and HI titre against ND antigen reached the peak in the third month post vaccination in both groups of chickens. This result agree with Nedelciu and Sofei (1990) and Madkour et al. (2001) who mentioned that there was no significant differences in antibody titres between the chicken groups received trivalent or monovalent ND oil emulsion vaccines.

Results tabulated in table (5 and 6) of EDS showed that the SNT titre and HI antibody titre increased gradually till the 4th month post vaccination reaching maximum titre with no significant difference between groups vaccinated by trivalent vaccine or the monovalent vaccine. These results agreed with those of Wu Yan Gong et al. (1994) and Hala et al. (2002).

Table (7) showed that the trivalent prepared oil emulsion vaccine when applied in the field gave a high immunological response as well as the monovalent vaccines. Normal equal mortality rate with no P/M lesion of any of the ND, IB and EDS diseases and no viruses reisolated in ECE. The egg production in trivalent vaccinated group is slightly similar as the monovalent vaccinated group which means that the prepared trivalent inactivated vaccine is potent, safe and gave an effective field protection against IB, ND and EDS viruses at the same time.

As a conclusion, we advice using of the trivalent vaccine is highly recommended to save time, efforts, costs and reduce number of vaccinations which constitute stress factors on chickens.

Table (1): Mean neutralizing antibody titres (log2x) against IBV in sera collected from vaccinated chickens

Groups	Titre of SNT								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	7	7	8	9	8	8	9	9	9
Group (2)	7	8	8	9	9	8	9	9	8

Group (1): Chicken vaccinated with trivalent prepared vaccine.

Group (2): Chicken vaccinated with monovalent IB vaccine

Table (2): Mean ELISA antibody titres (log2x) against IBV in sera collected from vaccinated chickens

Groups	Titre of SNT								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	1306	1460	3587	5596	5396	4410	4910	4150	3139
Group (2)	1311	1426	3591	5699	5661	4416	4902	4154	3111

Group (1): Chicken vaccinated with trivalent prepared vaccine.
Group (2): Chicken vaccinated with monovalent IB vaccine
Control positive serum = 290
Control negative serum = 65

Table (3): Mean neutralizing antibody titres (log2x) against NDV in sera collected from vaccinated chickens

Groups	Titre of HI								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	7	7	9	9	8	9	9	9	8
Group (2)	7	8	9	9	9	9	8	8	7

Group (1): Chicken vaccinated with trivalent prepared vaccine.
Group (2): Chicken vaccinated with monovalent ND, IB, EDS vaccines

Table (4): Mean haemagglutination inhibition antibody titres (log2x) of sera from chickens vaccinated with NDV vaccine

Groups	Titre of HI								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	8	10	11	11	11	10	11	10	9
Group (2)	8	10	11	11	10	10	10	9	9

Group (1): Chicken vaccinated with trivalent prepared vaccine.
Group (2): Chicken vaccinated with monovalent ND, IB, EDS vaccines

Table (5): Mean neutralizing antibody titres (log2x) against EDS in sera collected from vaccinated chickens

Groups	SNT titres								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	7	7	8	9	9	9	9	9	8
Group (2)	7	8	8	9	8	9	8	9	8

Group (1): Chicken vaccinated with trivalent prepared vaccine.

Group (2): Chicken vaccinated with monovalent IB, NDV, EDS vaccines

Table (6): Mean haemagglutination inhibition antibody titres (log2x) of sera from chickens vaccinated with EDS vaccine

Groups	Titre of HI								
	Months Post Vaccination								
	1	2	3	4	5	6	7	8	9
Group (1)	8	9	9	11	10	10	9	9	9
Group (2)	8	9	10	11	10	9	9	10	9

Group (1): Chicken vaccinated with trivalent prepared vaccine.

Group (2): Chicken vaccinated with monovalent IB, NDV, EDS vaccines Control positive serum = 290

Control negative serum = 65

Table (7): Data of vaccinated breeder farms in field application of trtivalent prepared locally oil emulsion vaccine and monovalent ND, IB, EDS vaccines.

Age (days)	Mortality %		P/M lesion		Reisolation in ECE		Medication used	Egg production/eggs	
	Group (1)	Group (2)	Group (1)	Group (2)	Group (1)	Group (2)		Group (1)	Group (2)
120	1	1.5-2	No specific ND, IB or EDS P/M lesions		-ve	-ve	H selenium, AD_3E, solurinal and water	2	2
130	0.5	0.5			-ve	-ve		57	55
150	0.05	0.2			-ve	-ve		2895	2890
180	0.05	0.05			-ve	-ve		4260	4199
210	7-8.5	7-8.5			-ve	-ve		4160	4105
240	1-1.5	1-1.5			-ve	-ve		4630	3320
270	0.05	0.05			-ve	-ve		3960	3900
300	0.05	0.05			-ve	-ve		3990	3997
330	0.2	0.05			-ve	-ve		4020	4022
344	0.05	0.05			-ve	-ve		3690	3680

Group (1): Chicken vaccinated with trivalent prepared vaccine.

Group (2): Chicken vaccinated with monovalent vaccines

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