STUDIES ON PREPARATION OF BIVALENT INACTIVATED OIL-EMULSION VACCINE AGAINST NEWCASTLE AND INFECTIOUS CORYZA DISEASES

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Abstract

Bivalent inactivated oil-emulsion vaccine against Newcastle disease virus (NDV) and infectious coryza disease was prepared. Immunological response to the vaccine was determined in susceptible birds by Heamagglutination-inhibition (HI), Tube agglutination antibody titer for infectious coryza, Lymphocyte transformation and challenge test. The results obtained indicated that the prepared vaccine gave high immune response and good protection against infection.

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, by causing exacerbation in dual or multiple infections with other organisms . Newcastle disease virus causes great economic losses due to high rate of mortality, reduction of meat and drop in egg production (Alexander, *et al.* 1985).

Infectious coryza is a serious world-wide disease of upper respiratory tract of chicken caused by *haemophilus paragallinarum*. This disease results in an increase in culling and a major reduction in egg production in layers (10-40%) (Vanzyl, *et al.* 2001). Avian respiratory diseases due to mixed infections by various organisms are very common and show more sever symptoms than disease due to single infection. Therefore, use of a mixed vaccine is preferable to that of a single agent vaccine for prevention of the common avian respiratory disease. Also, combined vaccines have the advantages of reducing vaccination expenses and saving time and labour costs. Many authors as Otsuki and Iritani (1974), Gergis *et al.* (1994), Xie and Stone (1990) prepared oil-emulision vaccines with single or mixed antigens of *pasteurella multocida*, *haemophilus paragallinarum*, Newcastle disease virus (NDV), infectious bronchitis virus (IB), Egg Drop syndrome (EDS), Avian influenza (AI) and fowl pox.

So, the aim of this work was preparation of a new efficient mixed vaccine of inactivated Newcastle disease (NDV) and inactivated *Haemophilus paragallinarum* to protect the birds against both diseases at the same time.

MATERIALS AND METHODS

1-Antigens

1.1. Newcastle disease virus (NDV)

NDV Lasota strain was supplied by the central veterinary laboratory, Weybridge, England.

1.2. *Haemophilus paragallinarum* strains

W strain (Serovar A), Modesto strain (Serovar C)-0222 strain (Serovar B) and a locally isolated strain (serovar A) of *Haemophilus paragallinarum* were used.

2- Embryonated eggs

Nine-eleven days old embryonated chickens (specific pathogen free, Egg production farm, Nile SPF eggs, Koum Oshiem, Fayoum, Egypt). Were used for propagation, preparation and titration of the prepared batches of NDV.

3- Experimental chicks

Two-hundred , one day old mixed sex commercial Hubhard chicks were supplied by (Ministry of Agriculture). The chicks were reared under complete hygienic measures in isolated and disinfected wire floored cages and fed commercial broiler ration.

4- Virus propagation

NDV was propagated in embryonated chicken eggs according to Allan *et al.,* (1973). The obtained virus was titrated according to the standard methods in FAO publication (1978). It had a titre of 10^{11} EID_{50} /ml.

5- *Haemophilus paragallinarum* bacterin preparation

Haemophilus paragallinarum bacterian was prepared according to method applied by Blackall *et al.* (1992)

6- Inactivation of virus

Inactivation of NDV carried out using formalin in a final concentration of 0.1% of the total volume for NDV vaccine.

7- Vaccines preparation

The monovalent oil-emulsion vaccine against NDV and *Haemophilus paragallinarum*, as well as, the bivalent vaccine were prepared according to Stone *et al.* (1978) with aqueous to oil ratio (1: 3). The prepared vaccines were adjusted to contain 10^9 EID_{50} for NDV and $3.96x10^8$ CFU /ml of W strain, $3.93x10^8$ CFU/ml of Modestostrain and $3.96x10^8$ CFU/ml of 0222 strain of *Haemophilus paragallinarum*.

8- Quality control

The prepared vaccines were subjected for quality control measures as described by Stone *et al.* (1979).

9- Experimental design

Two hundred one-day-old chicks were reared till they became 4 weeks old. The chicks were divided into 4 groups (50 chicks for each group).

Group (1): vaccinated with the prepared Monovalent oil-emulsion NDV vaccine.

Group (2): vaccinated with the monovalent oil-emulsion infectious coryza vaccine.

Group (3): vaccinated with Bivalent (NDV+infectious coryza) oil-emulsion vaccine

Group (4): Non-vaccinated control.

All chickens were vaccinated at 4 weeks old through I/M injection in breast muscle with 0.5ml from each vaccine.

Ten random blood samples were collected weekly from each group for 12 weeks post-vaccination. Sera were separated, collected and stored at 20°C until used for detection of corresponding antibodies against ND and *Haemophilus paragallinarum*.

10. Cellular immunity assay

It was estimated by blood lymphocyte blast genesis (Lucy, 1974 and 1977) and identified by (Charles *et al.*, 1978), Evaluation of the test using Tetrazolium calorimetric assay according to (Mossman, 1983).

11. Haemagglutination Inhibition test (HI) was used for estimating the haemagglutinating inhibiting antibodies against ND according to Majujabe and Hitchner, (1977).

12. Tube agglutination and Haemagglutination inhibition (HI) tests were used for detection of antibody titer for *Haemophilus paragallinarum* according to (Iritani *et al.*, 1977 and yamaguchi *et al.* 1989).

13- Challenge Test Three weeks post-vaccination 30 chicks of group 2 which received monovalent infectious coryza and 30 chicks of group 4 that were vaccinated with bivalent (ND+infectious coryza) vaccine, as well as, 30 chicks of group 4 control unvaccinated were challenged by inoculation of infraorbital sinus with 0.2 ml (approximately 10^8 C.F.U)/bird of 16-18 hours broth culture of W, Modest and 0222 strains of *Haemophilus paragallinarum*. All chicks were examined daily for clinical signs of infectious coryza. (Kume *et al.*, 1980 and Blackall *et al.*, 1992).

RESULTS AND DISCUSSION

The results illustrated in Table 1 indicated that tube agglutination antibody titer in chicken sera against either monovalent or Bivalent infectious coryza vaccines reached its Peak at 11-12 weeks post-vaccination, while, peak of HI antibody titer was

detected at 9 weeks post vaccination against both monovalent and Bivalent infectious coryza vaccines as shown in Tables 2. These results agreed with those mentioned by Kamal (1996). From results obtained in Tables 1 and 2, it was clear that there was no difference between antibody titres in chicken sera as detected by tube agglutination or HI test against either infectious coryza in separate or combined form (IC+ND). The results of challenge test in Table 3. supported these findings. Also, the results of cell-mediated immune response as represented in Table 5. come parallel with these findings. So, the locally prepared combined vaccine (ND+infectious coryza) can protect chicken against two disease without interference in the immune response against both diseases. These findings come to be confirmed by those of Gergis *et al.* (1994), who used fowl cholera vaccine in combined with other viral vaccines as NDV, IBV, Fowl pox and EDSV. They stated that there was no interference between bacterial and viral inactivated antigens in the immune response of vaccinated fowl to each other.

Table 1. Average antibody titer of *Haemophilus Paragallinarum* in chicken sera vaccinated with infectious coryza vaccine either alone or in combined form.

Weeks post-vacc.	No. of sera samples	G.M. of agglutination antibody titer of chicken sera using					
		W Strain (Serovar A) ant.		Modesto strain (Serovar C) Ant.		0222 strain (Serovar B) Ant.	
		for monovalent vaccine	for combined vaccine	for monovalent vaccine	for combined vaccine	for monovalent vaccine	for combined vaccine
Pre-vacc	10	0		0		0	
1 week	10	14.92	12.99	12.12	12.12	11.31	11.31
2 week	10	18.37	14.92	16.00	13.92	11.31	12.12
3 week	10	21.11	19.69	19.69	18.37	13.92	12.99
4 week	10	24.25	22.62	22.62	21.11	17.14	16.00
5 week	10	25.99	24.25	24.25	22.62	19.69	19.69
6 week	10	29.58	24.25	25.99	22.62	21.11	21.11
7 week	10	29.85	27.85	27.85	24.25	24.25	22.62
8 week	10	34.29	32.00	32.00	27.85	27.85	25.99
9 week	10	36.75	34.29	32.00	29.85	27.85	29.85
10 week	10	39.39	36.75	36.75	32.00	32.00	29.85
11 week	10	42.22	39.39	39.39	34.29	34.29	32.00
12 week	10	42.22	36.75	39.39	34.29	36.75	32.00

Table 2. Humoral immune response of chicken vaccinated with infectious Coryza vaccines either alone or in combined form.

Weeks Post-Vacc.	No. Of Sera Samples	G. M. of HI antibody titer of chicken sera using					
		W Strain (Serovar A) ant.		Modesto Strain (Serovar C) ant.		0222 Strain (Serovar B) ant.	
		For monovalent vaccine	For combined vaccine	For monovalent vaccine	For combined vaccine	For monovalent vaccine	For combined vaccine
Pre-vacc	10	0	0	0	0	0	0
1 week	10	13.92	12.99	12.99	12.12	10.55	12.12
2 week	10	13.92	12.99	13.92	12.99	11.31	12.12
3 week	10	14.92	14.92	14.92	13.92	12.12	12.99
4 week	10	17.14	16.00	16.00	14.92	13.92	13.92
5 week	10	21.11	19.69	18.37	17.14	13.92	14.92
6 week	10	24.25	22.62	21.11	19.69	16.00	16.00
7 week	10	27.85	25.99	24.25	22.62	19.69	18.37
8 week	10	32.00	29.85	27.85	25.99	27.85	24.25
9 week	10	32.00	29.85	29.85	27.85	27.85	25.99
10 week	10	29.85	25.99	25.99	24.25	24.25	22.62
11 week	10	29.85	24.25	24.25	22.62	24.25	22.62
12 week	10	27.85	24.25	24.25	21.11	21.11	21.11

HI : Haemagglutination Inhibition St : Strain
G.M : Geometric Mean Ant : Antigen Pre-Vacc.: Prevaccionation.

Table 3. Result of challenge test of chicken vaccinated with either monovalent infectious Coryza or bivalent (ND+ Infectious Coryza) Vaccines.

Chicken vaccinated with	Strain used in challenge test	No. of chicken	No. of protected chicken	No. of chicken have clinical signs	Protection rate %
Monovalent infectious coryza vaccine	W St. (Serovar A)	10	8	2	80
	Modesto St.(Serovar C)	10	7	3	70
	0222 St. (Serovar B)	10	7	3	70
Bivalent infectious coryza vaccine (ND+IC)	W St. (Serovar A)	10	7	3	70
	Modesto St.(Serovar C)	10	7	3	70
	0222 St. (Serovar B)	10	7	3	70
Control unvaccinated chicken	W St. (Serovar A)	10	0	10	0
	Modesto St.(Serovar C)	10	0	10	0
	0222 St. (Serovar B)	10	0	10	0

IC : infectious coryza

Table 4. The average log_2 HI antibodies titer to NDV in vaccinated group.

Chicken group vaccinated with	Weeks post- vaccination								
	1	2	3	4	5	6	8	10	12
Monovalent ND vaccine	6	8	11	10	9	9	9	8	8
Bivalent (ND+IC) vaccine	5	7	10	11	9	9	8	8	8
Control unvaccinated chicken	0	0	0	0	0	0	0	0	0

Table 5. Evaluation of cell-mediated immune response of vaccínated groups by lymphocyte transformation expressed by optical density.

Chicken group vaccinated with	Weeks post - vaccination			
	1st week	2nd week	3rd week	4th week
Bivalent vaccine Ic+NDV	0.304	0.438	0.45	0.63
Monovalent ND vaccine	0.241	0.466	0.42	0.40
Monovalent infectious coryza vaccine	0.242	0.619	0.47	0.44
Control unvaccinated chicken	0.21	0.196	0.191	0.188

Ic = Infectious coryza
ND = Newcastle disease

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دراسات لتحضير لقاح ثنائي مثبط زيتي ضد مرض النيوكاسل وزكام الطيور المعدي

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تم تحضير لقاح ثنائي زيتي مزدوج لفيروس النيوكاسل وزكام الطيور المعدي وتم تقييمه سيرولوجيا لاختبار كفاءة اللقاح المحضر الذي أثبت كفاءة عالية عند اختباره معمليا وعند اختبار التحدى .