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TRIALS FOR PREPARATION OF BIVALENT INACTIVATED OIL VACCINE AGAINST EGG DROP SYNDROME AND INFECTIOUS CORYZA DISEASES IN LAYING HENS

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SUMMARY

Twelve inactivated oil adjuvanted vaccines (4 for EDS virus, 4 for Haemophilus paragallinarum and 4 for bivalent vaccines) were prepared by using different ratios of Tween 80, span 80. Hydrophile-lipophile Balance (HLB) was determined for all vaccines. It was found that combined vaccine protected chicken against infectious coryza and egg drop syndrome (EDS) diseases with no interference between them in immune response of chicken. The vaccines that have HLB of 4.98 resulted in higher antibody titers more than those have 5.4, 6.01 and 6.58 HLB respectively.

INTRODUCTION

Infectious coryza is an acute respiratory tract disease of chicken caused by Haemophilus paragallinarum, the greatest economic losses of infectious coryza (IC) disease is a marked reduction (10-14%) in egg production (Blackall and Matsumoto, 2003). Egg drop syndrome (EDS-76) is a disease of laying hens characterized by a sudden and frequently large drop in egg production with the laying of soft-shelled eggs (Holmes et al., 1989).

Combined vaccine is an important approach to poultry industry to combat the risk of several diseases in poultry for which adequate vaccination is available. Combined vaccines save time and labor costs as well as reducing stress reactions especially in laying hens. Many authors as Winterfield (1982), Otsuki and Iritani (1974), Gergis et al. (1994), Samira et al. (1995), Khodeir et al. (1999) and Xie and Stone (1990) prepared oil-emulsions vaccines with single or mixed anti-

linarum, Newcastle disease virus (NDV), avian influenza virus (AIV), infectious bronchitis virus (IBV), egg drop syndrome virus (EDSV), and fowl pox.

Oil-emulsion killed vaccines are a critical component of broiler, breeder and layer vaccination programs, inactivated bacterial and viral vaccines are commonly administered in this form to provide longer duration of immunity and improve antibody titre for breeders to provide protective antibodies to the broiler or layer progeny.

Oil-phase emulsifier (e.g. span 80) and water-phase emulsifier (e.g. Tween 80) have been shown to improve efficacy, lower viscosity, increase stability and reduce undesirable side effects of oil-emulsion vaccines (Stone et al., 1983 and Pokric et al., 1993). The hydrophile-lipophile balance (HLB) of a mixture of surfactants is the weighted average of each individual surfactant (HLB) (Rosen, 1978) and can influence the release of solutes from the water-in-oil emulsion (Chiejiha and Sewell, 1974). The relationship between antigen release rate constant (K), HLB number, antigen/oil (A/O) ratio of oil adjuvant infectious coryza vaccines and chicken immune response was studied by Fukanoki et al. (2000). So, the aim of the present study is to prepare and evaluate a combined oil adjuvant vaccine for EDS and IC by using different ratios of Tween 80 and span 80.

MATERIAL AND METHODS

1. Embryos:

Embryonated duck eggs obtained from the United Company for Poultry Production were used for propagation of EDS virus and testing of its complete inactivation.

2. Experimental chicken:

Five hundred and ten (510) Hubbard chicks (one-day-old) were used in this study. All chicks were reared under hygienic measures and checked just before the experiment for absence of avian infectious agents (bacteria or virus) and EDS or Haemophilus paragallinarum antibodies.

3. Vaccines preparation:

a. EDS-76 virus:

The vaccine product Code PA, 0081 was propagated in embryonated duck eggs and its titre was adjusted to be 10^7 EID_{50}/ml according to method applied by Allan et al. (1973) and Awad et al. (2001).

b. Haemophilus paragallinarum:

Standard W (serovar A), Modesto (serovar C), 0222 (serovar B) strains and a locally isolated strain (serovar A) were used in bacterins preparation according to Blackall et al. (1992).

Both antigens were used for preparation of oil adjuvant monovalent and bivalent vaccines using liquid paraffin oil and different ratios of water-emulsifier (Tween 80) and oil-emulsifier

(span 80), HLB for each vaccine was determined through the equation of Fukanoki et al. (2000).

$$\frac{a \times 3.7}{a+b} + \frac{b \times 15.0}{a+b}$$

Where:

"a" and "b" are amounts of oil emulsifier and water emulsifier as shown in table (1).

4. Vaccination and challenge exposure:

As shown in Table (2), 12 groups of chicken were vaccinated at six-week old subcutaneously (0.5ml/bird) at dorsum-back of the neck with one dose of different prepared vaccines.

Three weeks post-vaccination, (30) chicken of each vaccinated group with IC vaccines and combined vaccines as well as 30 chicken of control unvaccinated group were challenged by inoculation of infraorbital sinus with 0.2ml (10^8 CFU approximately/bird of 16-18 hours broth culture of Haemophilus paragallinarum W, Modesto and 0222 strains. All chicken were examined daily for 7-days for typical clinical signs of infectious coryza (Kume et al., 1980, and Blackall et al., 1992).

5. Serological tests:

Serum samples were taken from all chicken groups prevaccination and every two weeks post vaccination for detection of antibody titre of IC vaccines by using tube-agglutination, haemagglutination inhibition (HI) according to Iritani et al. (1977) and Yamaguchi et al. (1989) and EDS titres by using HI, SN and ELISA tests according to Rossiter et al. (1985).

RESULTS AND DISCUSSION

The results illustrated in Tables (3, 4, 6 and 8) indicated that there was no difference between antibody titres in chicken sera as detected by tube-agglutination, HI and ELISA tests against either IC or EDS vaccines separately or in combined form (IC+EDS), results of challenge test as in Table (5) supported these findings, so the locally prepared combined vaccine protects chicken without interference in the immune response against both diseases. These findings are in agreement with Winterfield (1982); Gergis et al. (1994); Samira et al. (1995) and Khodeir et al. (1999) who used fowl cholera vaccine in combination with other viral vaccines as NDV, IBV, fowl pox and EDS. They stated that there was no interference between bacterial and viral inactivated antigens in the immune response of vaccinated fowl to each other.

Dealing with the results of Table (7), it was clear that bivalent (EDS+IC) vaccines yielded SN titre higher than monovalent EDS vaccine from 1st to 12th weeks post vaccination. These findings are parallel with those of Thornton and Muskett (1975) and Xie and Stone (1990) as they found that IBV component in live attenuated combined NDV/IBV vaccine may interfere with the ability

Table (1): Different formulations of vaccines used

Type of vaccine	Vaccine No.	Tween 80	Span 80	HLB
IC	1	3%	10%	4.98
	2	3%	5%	6.01
	3	4%	10%	5.40
	4	4%	5 %	6.58
EDS	5	3%	10%	4.98
	6	3%	5%	6.01
	7	4%	10%	5.40
	8	4%	5%	6.58
IC+EDS (Combined)	9	3%	10%	4.98
	10	3%	5%	6.01
	11	4%	10%	5.40
	12	4%	5%	6.58

IC: Infectious Coryza

EDS: Egg Drop Syndrome.

HLB: Hydrophile - Lipophile Balance

Table (2): Groups of vaccinated chicken with different vaccines

Vaccine No.	Group of chicken	No. of chicken
1	1	40
2	2	40
3	3	40
4	4	40
5	5	40
6	6	40
7	7	40
8	8	40
9	9	40
10	10	40
11	11	40
12	12	40
Control unvaccinated	13	30

Table (3): Average antibody titre of Haemophilus paragallinarum in chicken sera vaccinated with different infectious coryza vaccines by using tube agglutination test

Chicken group	Type of vaccine			Geometric mean of agglutination antibody titer of chicken sera using:																		
				"W" strain (Serovar A) ant.							Modesto strain (Serovar C) ant.							0222 strain (Serovar B) ant.						
	No.	Tween %/ Span %	HLB	Serum samples (10) taken post vaccination at:							Serum samples (10) taken post vaccination at:							Serum samples (10) taken post vaccination at:						
				Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W	Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W	Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W
1	1	3/10	4.98	0	19.69	21.11	25.99	29.85	34.92	32.0	0	17.14	18.37	21.11	22.62	27.85	25.99	0	13.92	16.0	17.14	19.69	24.25	21.11
2	2	3/5	6.01	0	14.92	16.00	18.37	21.11	27.85	25.99	0	13.92	14.92	16.00	17.14	22.62	19.69	0	12.12	12.99	13.92	16.00	19.69	17.14
3	3	4/10	5.40	0	16.00	18.37	21.11	22.62	29.85	27.85	0	14.92	17.14	18.37	19.69	24.25	22.62	0	12.99	13.92	16.00	17.14	21.11	18.37
4	4	4/5	6.58	0	12.12	14.92	16.00	19.69	22.62	18.37	0	10.55	11.31	12.99	14.92	18.37	16.00	0	9.84	10.55	12.12	13.92	16.00	14.92
9	9	3/10	4.98	0	18.37	21.11	24.25	29.85	32.00	34.92	0	18.37	19.69	21.11	24.25	25.99	25.99	0	13.92	14.92	17.14	18.37	25.99	22.62
10	10	3/5	6.01	0	13.92	16.00	17.14	19.69	25.99	24.25	0	12.99	13.92	16.00	17.14	21.11	18.37	0	11.31	12.12	13.92	14.92	18.37	17.14
11	11	4/10	5.40	0	16.00	17.14	21.11	21.11	27.85	25.99	0	13.92	19.69	18.37	21.11	24.25	25.99	0	12.99	13.92	14.92	16.00	19.69	18.37
12	12	4/5	6.58	0	11.31	14.92	16.00	18.37	21.11	18.37	0	11.31	12.12	12.99	14.92	17.14	16.00	0	10.55	11.31	12.12	12.99	14.92	13.92

Group (1): Vaccinated with monovalent IC vaccine (HLB 4.98).
Group (2): Vaccinated with monovalent IC vaccine (HLB 6.01).
Group (3): Vaccinated with monovalent IC vaccine (HLB 5.40).
Group (4): Vaccinated with monovalent IC vaccine (HLB 6.58).
Group (9): Vaccinated with combined (IC+EDS) vaccine (HLB 4.98).
Group (10): Vaccinated with combined (IC+EDS) vaccine (HLB 6.01).
Group (11): Vaccinated with combined (IC+EDS) vaccine (HLB 5.40).
Group (12): Vaccinated with combined (IC+EDS) vaccine (HLB 6.58).

Table (4): Average antibody titre of Haemophilus paragallinarum in chicken sera vaccinated with different infectious coryza vaccines by using haemagglutination inhibition (HI) test

Chicken group No.	Type of vaccine: Tween % / Span %	HLB	Geometric mean of agglutination antibody titer of chicken sera using: "W" strain (Serovar A) ant. Serum samples (10) taken post vaccination at: Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W	Modesto strain (Serovar C) ant. Serum samples (10) taken post vaccination at: Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W	0222 strain (Serovar B) ant. Serum samples (10) taken post vaccination at: Pre-vac.	2 W	4 W	6 W	8 W	10 W	12 W
1	1 3/10	4.98	0	19.69	25.99	32.00	29.85	27.85	25.99	0	16.00	21.11	27.85	25.99	22.62	21.11	0	14.92	21.11	24.25	22.62	21.11	18.37
2	2 3/5	6.01	0	16.00	19.69	25.99	22.62	21.11	18.37	0	12.99	16.00	24.25	21.11	18.37	16.00	0	12.12	17.14	18.37	16.00	13.92	12.99
3	3 4/10	5.40	0	17.14	22.62	27.85	25.99	24.25	21.11	0	14.92	18.37	25.99	22.62	21.11	19.69	0	12.99	18.37	21.11	19.69	17.14	16.00
4	4 4/5	6.58	0	13.92	16.00	21.11	18.37	17.14	14.92	0	11.31	14.92	19.69	18.37	16.00	13.92	0	9.84	13.92	16.00	14.92	12.99	10.55
9	9 3/10	4.98	0	18.37	24.25	32.00	27.85	25.99	24.25	0	16.00	21.11	25.99	24.25	22.62	21.11	0	13.92	19.69	24.25	22.62	21.11	17.14
10	10 3/5	6.01	0	16.00	18.37	24.25	22.62	21.11	17.14	0	12.12	16.00	22.62	21.11	17.14	16.00	0	11.31	14.92	17.14	16.00	13.92	12.99
11	11 4/10	5.40	0	17.14	21.11	25.99	24.25	22.62	21.11	0	14.92	17.14	24.25	22.62	21.11	19.69	0	12.12	17.14	19.69	18.37	17.14	16.00
12	12 4/5	6.58	0	12.99	14.92	19.69	18.37	17.14	14.92	0	10.55	13.92	18.37	17.14	16.00	13.92	0	9.84	12.99	14.92	13.92	12.99	11.31

Table (5): Results of challenge test of chicken vaccinated with different infectious cortza vaccines

Chicken group	Strain used in challenge	No. of Chicken	No. of protected chicken	No. of chicken have clinical signs	Protection rate %
1	W(serovar A)	10	8	2	80
	Modesto (serovar C)	10	8	2	80
	0222 (serovar B)	10	7	3	70
2	W(serovar A)	10	7	3	70
	Modesto (serovar C)	10	7	3	70
	0222 (serovar B)	10	6	4	60
3	W(serovar A)	10	7	3	70
	Modesto (serovar C)	10	6	4	60
	0222 (serovar B)	10	6	4	60
4	W(serovar A)	10	6	4	60
	Modesto (serovar C)	10	6	4	60
	0222 (serovar B)	10	6	4	60
9	W(serovar A)	10	7	3	70
	Modesto (serovar C)	10	8	2	80
	0222 (serovar B)	10	7	3	70
10	W(serovar A)	10	7	3	70
	Modesto (serovar C)	10	7	3	70
	0222 (serovar B)	10	7	3	70
11	W(serovar A)	10	6	4	60
	Modesto (serovar C)	10	7	3	70
	0222 (serovar B)	10	6	4	60
12	W(serovar A)	10	6	4	60
	Modesto (serovar C)	10	6	4	60
	0222 (serovar B)	10	6	4	60
13	W(serovar A)	10	0	10	0
	Modesto (serovar C)	10	0	10	0
	0222 (serovar B)	10	0	10	0

* Control unvaccinated group.

Table (6) : Humoral immune response of chicken vaccinated with different egg drop syndrome vaccines as determined by HI test

Chicken groups	Types of vaccine			*GM log2 haemagglutinating-inhibition antibody titre of chicken sera						
				Serum samples taken post vaccination at:						
	No.	Tween %/ Span %	HLB	Prevac.	2W	4W	6W	8W	10W	12W
5	5	3/10	4.98	0**	2.9	4.9	6.9	6.4	6.2	6.7
6	6	3/5	6.01	0	1.5	3.1	3.5	4.9	4.6	4.9
7	7	4/10	5.40	0	2.4	3.5	4.7	5.7	5.4	5.1
8	8	4/5	6.58	0	1.5	2.3	2.7	3.1	4.3	4.7
9	9	3/10	4.98	0	2.7	6.2	6.7	5.9	6.4	6.9
10	10	3/5	6.01	0	1.8	4.6	4.8	4.5	4.9	5.4
11	11	4/10	5.40	0	2.6	5.8	5.6	5.9	5.2	5.9
12	12	4/5	6.58	0	1.9	2.7	3.6	4.4	3.1	4.7

Group (5): Vaccinated with monavalent EDS vaccine (HLB 4.98).
Group (6): Vaccinated with monavalent EDS vaccine (HLB 6.01).
Group (7): Vaccinated with monavalent EDS vaccine (HLB 5.40).
Group (8): Vaccinated with monavalent EDS vaccine (HLB 6.58).
* GM log2: Geometric Mean of HI titre Prevace: Precaccination
** 0= No antibody against EDS virus was detected in chicken before vaccination

Table (7) : Humoral immune response of chicken vaccinated with different egg drop syndrome vaccines as determined by serum neutralization test (SNT).

Chicken groups	Types of vaccine			Arithmetic mean of SNT titre of chicken sera						
				Serum samples taken post vaccination at:						
	No.	Tween %/ Span %	HLB	Prevac.	2W	4W	6W	8W	10W	12W
5	5	3/10	4.98	0**	24	96	96	129	46	160
6	6	3/5	6.01	0	12	24	32	48	32	40
7	7	4/10	5.40	0	20	64	80	96	49	80
8	8	4/5	6.58	0	20	24	49	49	32	24
9	9	3/10	4.98	0	46	128	192	256	96	96
10	10	3/5	6.01	0	24	32	64	64	49	64
11	11	4/10	5.40	0	24	96	128	128	192	96
12	12	4/5	6.58	0	12	32	32	64	48	32

** 0= No antibody against EDS virus was detected in chicken before vaccination

Table (8) : Immune response of chicken vaccinated with different egg drop syndrome vaccines as determined by ELISA test.

Chicken groups	Types of vaccine			Arithmetic titre in sera of vaccinated chicken						
				Serum samples taken post vaccination at:						
	No.	Tween %/ Span %	HLB	Prevac.	2W	4W	6W	8W	10W	12W
5	5	3/10	4.98	298	2458	3650	4859	4738	4689	4750
6	6	3/5	6.01	277	2285	2788	3966	3865	2975	2867
7	7	4/10	5.40	308	2336	3387	4528	4698	3496	3630
8	8	4/5	6.58	320	2158	2640	2556	2493	2683	2780
9	9	3/10	4.98	371	2580	3460	4550	4600	4850	4260
10	10	3/5	6.01	381	2240	3260	3290	3320	3590	3480
11	11	4/10	5.40	345	2260	3290	4360	4450	4730	4230
12	12	4/5	6.58	409	2230	2220	2280	3340	2360	3390

of chicken to respond to NDV component. They also added that monovalent inactivated NDV vaccines are the most efficacious when used alone and the polyvalent vaccine perform better when made up of combined monovalent vaccines rather than mixed antigens that are then emulsified. Also, Otsuki and Iritani (1974), Nedelciu and Safei (1990) and Awad et al. (2001) stated that bivalent (NDV+IBV), trivalent (NDV + IBV + EDSV or NDV + IBV + Haemophilus paragallinarum) and tetravalent (NDV+IBV+EDSV+fowl cholera) inactivated vaccines showed higher immunogenicity than a single vaccine.

From the obtained results in this study, concerning the HLB, it was noticed that the prepared vaccine either in single or combined form with HLB of 4.98 (Tween 3%, Span 10%) resulted in highest antibody titres followed by vaccines of HLB 5.4, 6.01 and 6.58 respectively from the 1st to 12th week post vaccination as determined by HI, tube agglutination, SN and ELISA tests. These findings come in accordance with Fukanoki et al. (2000) who found that IC vaccine of HLB 4.8 maintained high HI antibody titre even at 10 week post vaccination due to release rate constant of this vaccine is smaller in value than that at an HLB of 6.0 (The value of K is the release rate constant). Furthermore, the vaccine of small value of (K) showed a stronger and more prolonged immune response due to slow release of antigen from the formulation.

So, it could be concluded that the locally prepared combined inactivated oil adjuvant vaccine (EDS+IC) is safe and potent and it is preferable to be prepared with 3/10 ratio (Tween/span) to give HLB 4.98 which is the best for immune response.

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