# Comparative Efficacy of Montanide ISA 50, Saponin, and Calcium Phosphate Adjuvants for Infectious Coryza Vaccines Kamal, O: E.

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Abstract

Four types of infectious coryza vaccines were prepared in this study using liquid paraffin oil (30/70), Montanide ISA 50 oil (1:1), Saponin (0.0004%) and Calcium phosphate (50%) adjuvants and evaluated in chicken (7-weeks old). The results showed that the use of Montanide ISA 50 in the preparation of infectious coryza vaccine as oil adjuvant produced higher immune responses and protection rates followed by those prepared with liquid paraffin oil and Calcium phosphate adjuvant yielded poor immune responses and low protection rates.

#### Introduction

The goal of vaccination is to stimulate strong, protective and long lasting immune responses to the administered antigens, for the achievement of these objectives, potent adjuvant and novel vaccine strategies are required to make the vaccine sufficiently immunogenic to initiate potent immune response (16 and 8). New generation adjuvants are designed to induce minimal side effects, enhance the duration of immune response, and concurrently stimulate humoral, cellular and mucosal immune responses, furthermore, an ideal adjuvant would be biodegradable, economical and simple to manufacture. (31).

We ter-in-oil emulsions have been widely used as immunopotentiating delivery systems for veterinary vaccine antigens. Recently, several studies reported that using of oil adjuvant of Montanide series (Seppic-France) as Montanide ISA 25, 50, 70, 206 and IMS 1113 resulted in an improvement of in mune response and those new oil formulations have favorable characteristics of lower viscosity, lower reactivity and higher potency (29; 4; 36; 1; 3; 24 and 35).

Saponin adjuvant have been widely used as an adjuvant for many years and have been included in several veterinary vaccines, it has the ability to modulate the cell-mediated immune system as well as to enhance antibodies production and has the advantage that only a low dose is needed for adjuvant activity (30). Saponin (Quil A) was used as an adjuvant for inactivated infectious coryza vaccine (33).

Ca'cium phosphate (CAP) is a novel adjuvant elicited little or no inflammation at the inoculation site and induced higher titer of neutralizing antibodies, additional benefits of CAP include, an insignificant IgE response which is an important advantage than injection of alum compounds and CAP is a natural constituent of the animal body, thus it is very well tolerated and absorbed (31 and 32). In Europe CAP has been used as adjuvant for immunity against diphtheria and tetanus antigens (34).

Infectious coryza is a worldwide disease of the upper respiratory tract of chicken caused by *Haemophilus paragallinarum*, the greatest economic losses of infectious coryza disease is a marked reduction (10-40%) in egg production (5).

The objective of this study was to assess the chicken immune response against different formulations of infectious coryza vaccines by using mineral oil, Montanide ISA 50 oil, calcium phosphate and saponin adjuvents.

#### Materials and Methods

#### - Bacterial strains:-

Standard W (serovar A); Modesto (serovar C) and 0222 (serovar B) strain, and a locally isolated strain (serovar A) of *Haemphilus parag Illinarum* were used in this study for preparation of different infectious coryza vaccines.

## - Chicken:-

Or e hundred and ninety (190) white leg horn chicken (7 weeks old) were tested just before experiment to be sure that they were free from a vian

infectious agents (bacteria or viruses) and antibodies against *Haemophilus* paragallinarum; they are used for evaluation of the prepared vaccines.

- Adjavants:-
- Liquid paraffin and emulsifiers:-

Licuid paraffin (Whitrex 309, Total Co.), Span 80 (oil emulsifier-Sigma, Co.) and Tween 80 (water emulsifier-Sigma, Co.) were used for preparation of water-in-oil emulsion (W/O) vaccine.

- Montanide ISA 50 oil (Seppic-France):-
- It based on mannide oleate in mineral solution, render a water-in-oil emuls on (W/O).
- Saponin: (Riedel-deHaen, Sigma-Aldrich Laborchemikalien GmbH Germany).
- Calcium phosphate (CAP):-It composed of 12.5mM Calcium chloride (Winlab), 12.5 mM Dibasic sodium phosphate and 15.6 mM Sodium citrate (analar-Sigma), and mixing for preparation according to the method described by (32).
- Vaccines preparation:-

Four types of infectious coryza vaccines were prepared by using standard strains (W "serovar A ", Modesto "serovar C "and 0222 "serovar B ") as well as a locally isolated strain (serovar A) of *Haemohlus paragalinarum*, the cultures of these stains were prepared according to method of (7), and equal parts of each type were mixed together then used in vaccines preparation as following:

- Vaccine No. 1: oil adjuvant (W/O) infectious coryza vaccine, prepared in ratio of 30 / 70 using liquid paraffin, tween 80 and span 80 according to method of (39).
- Vaccine No. 2: Montanide ISA 50 oil adjuvant infectious coryza vaccine and was prepared by emulsifying aqueous phase containing antigen with Montanide ISA 50 in ratio of 1: 1 according to the manufacturer construction and the method of (20).
- Vaccine No. 3: Saponin adjuvant infectious coryza vaccine, prepared by adding purified saponin to the antigen to a final concentration of 0.0004 % (w/v) according to method of (33).

• Vaccine No. 4: Calcium phosphate (CAP) adjuvant infectious coryza vaccine, CAP was added in concentration of 50 % to the antigen, according to (32 and 15).

## - Experimental design:-

One hundred and ninety (190) white leg horn chicken (7 weeks old) were divided into five groups, group A, B, C, and D (40/each) vaccinated with infectious coryza vaccines No. 1, 2, 3, and 4 respectively (one dose, 0.5 ml/bird injected subcutaneously at dorsum-back of the neck) and group E (30 chicken) left as control non-vaccinated chicken. Three weeks post-vaccination, 30 chicken of each vaccinated group (A,B,C, and D) as well as, 30 chicken of control group (group E) were challenged by inoculation of infraorbital sinus with 0.2 ml (108 CFU approximately) for each kird of 16-18 hour broth culture of *Haemophilus paragalliarum* W, Modesto and 0222 strains (28 and 7). All chicken were kept under observation for ten days post challenge for presence of clinical signs of infectious coryza.

# - Serplogical tests:-

Ten serum samples were taken from the ten chicken left without challenge test from each vaccinated chicken group every two weeks post-vaccination until 16 weeks and tested for detection of antibody titers in chicken against different prepared infectious coryza vaccines by using tube agglutination and haemagglutination inhibition (HI) tests according to (19 and 40).

## Results and Discussion

Adjuvants used to improve the immune responses against antigens component of different vaccines by increasing the immunogenicity of weak antigens, enhancing speed and prolongation the duration of the immune response, modulating the antibody avidity, specificity, stimulating humoral and cell mediated immunity and decreasing dose of antigen in the vaccine to reduce costs. (38).

The results of tube agglutination and haemagglutination-inhibition tests on chicken sera vaccinated with liquid paraffin oil adjuvant infectious coryza vaccine (vaccine No.1) as shown in table (2 and 3) and protection rates (Table 1) indicated that this adjuvant gave high levels of antibody titers through out 16 weeks post vaccination, these findings comes 'parallel with that reported by (7; 23; 24 and 2), as they found that infectious coryza vaccine adjuvnated with liquid paraffin oil yielded high protection rates and antibody levels of chicken against infectious coryza disease. While using of Montanide ISA 50 oil adjuvant resulted in highest levels of antibody titers and protection rates (vaccine No. 2) as compared with the other adjuvants (liquid paraffin oil, Saponin and Calcium phosphate shown in tables (1, 2 and 3) these findings are in agreement with (22) who examined effect of dosage and the adjuvant in peptide vaccine derived from malarial parasite surface antigen, the malarial antibody levels in mice indica ed that Montanide ISA 50 was a superior adjuvant as compared with complete freund's adjuvant (CFA) and aluminum hydroxide gel adjuvant , also studies of (21) revealed that it should be useful to replace CFA with Montanide ISA 50 and (9), who found that Rota virus vaccines for calves prepa ed with Montanide ISA 50 oil were free flowing and less irritating to tissues, in addition (14 and 20), they found similar results. (3) explained this enhancement of Montanide oil adjuvants, was due to this new oil include new immunostimulant listed as GRAS substance which reported to elicit both humoral and cellular responses, several studies highlight the safety and superior adjuvant efficacy of Montanide series oils as Montanide ISA 71, 720, 51, 206, 25 (4; 1; 26; 12; 24 and 35).

Regarding using Saponin as adjuvant (vaccine No.3) the results showed that this vaccine could protect chicken to certain limit in spite of producing lowesi levels of antibody titers and protection rates in comparison with the other vaccines, these findings nearly similar with that obtained by several authors as (13; 11; 27; 30, and 37), when they used Saponin as adjuvant with different bacterial antigen as *E-coli* 018, *Streptococcus pneumonia*, and *Streptococcus bovis*, they found that Saponin adjuvant was effective and increasing antibody titers. On the contrary, results of this study disagree with that mentioned by (33), they found that Saponin in a killed

Haemophilus paragallinarum vaccine was inadequate but (6), found that aluiminium hydroxide gel plus Quil A (Saponin) as adjuvant in a killed Haemophilus paragallinarum vaccine gave greater protection, (10) explain this difference, they reported that the purity of a Saponin sample affected the immune response.

On the other hand, using of Calcium phosphate (CAP) adjuvant in infectious coryza vaccine (vaccine No. 4) showed that CAP was potent and efficient adjuvant induced higher levels of antibody titers and protection rates more than Saponin but less than liquid paraffin and Montanide ISA 50 oils adjuvants as shown in tables 1,2 and 3 with relative absence of side effects at the inoculation site, these results come in accordance with that reported by (15), who found that CAP in ratio of 50 % was more potent adjuvant than alum when used in inactivated Rift Valley Fever vaccines, as well as , with (32), they found that Herp's simplex virus type 2 (HSV-2) plus Calcium phosphate gave high antibody level at 6 weeks postvaccination and still high up to 14 weeks. Also, the clinical studies conducted in France described the use of CAP for secondary or booster immunization against diphtheria and tetanus(18), in addition to, (25 and 17), reported that CAP particles produced strong adjuvant effect, induced less immunoglobulin E (Ig E) than alum adjuvant and elicited only minimal local irritation in animal experiments.

Finally, the results of this study concluded that using Monanide ISA 50 oil adjuvant in infectious coryza vaccine was efficient and superior adjuvant followed by liquid paraffin and Calcium phosphate adjuvant, while using of Saporan as adjuvant in infectious coryza vaccine resulted in poor antibody responses and low protection rate

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Table (1): Results of challenge tests of chicken vaccinated with different infectious coryza vaccines.

Chicken Vaccine group type		Strain used in challenge	No. of chicken	No. of Protected Chicken	No. of chicken have clinical signs	Protection Rate %	
		w	10	7	3	70	
A	No. 1	Modesto	10	7	3	70	
		0222	10	6	4	60	
	No. 2	W	10	8	2	80	
В		Modesto	10	8	2	80	
		0222	10	7	3	70	
,	No. 3	W	10	5	- 5	50	
C		Modesto	10	5	5	50	
		0222	10	5	5	50	
	No. 4	W	10	6	6	60	
D		Modesto	10	6	6	60	
		0222	10	6	6	60	
	Control	W	10	0	10	0	
E		Modesto	10	0	10	0	
		0222	10	0	10	0	

<sup>-</sup> Vaccine No. 1:- liquid paraffir oil adjuvant infectious coryza vaccine.

Third Inter. Sci. Conf., 29 Jan.- 1 Feb./ 2009, Benha & Ras Sudr, Egypt

Fac. Vet. Med. (Moshtohor), Benha Univ

<sup>-</sup> Vaccine No. 2:- Montanide ISA 50 oil adjuvant infectious coryza vaccine

<sup>-</sup> Vaccine No. 3:- Saponin adjuvant infectious coryza vaccine.

<sup>-</sup> Vaccine No. 4:- Calcium phosphate (CAP) adjuvant infectious coryza vaccine

Table (2): Geometric mean of tube agglutination antibody titers in sera of chicken vaccinated with different infectious corvey vaccines.

St. Ag.	vaccine	No. of	Ch. g.	· Weeks post-vaccinaion									
				Pre.v	2 ,	4	6	8	10	12	14	16	
W st. Ag.	No.1	iG	À	0	19.69	24.25	27.85	32.06	36.75	35.59	34.29	29.85	
	No.2	10	В	0	29.85	34.29	36.75	42.22	55.71	51.98	42.22	39.39	
	No.3	10	c,	0	11.31	12.12	12.99	17.14	18.37	16.00	13.92	11.31	
	No.4	10	D	0	18.37	19.69	24.25	25.99	22.62	19.69	16.00	16.00	
Modosto. st Ag.	No.1	10	A	0	17.14	21.11	22.62	27.85	32.00	34.29	29.85	25.99	
	No.2	10	, B	0	24.25	27.85	32.00	39.39	42.22	42.22	36.75	34.29	
	No.3 >	- 10	С	0	10.55	12.12	12.99	16.00	17.14	14.92	12.99	10.55	
	No.4	10	D	0	16.00	17.14	21.11	22.62	19.69	18.37	16.00	14.50	
0222 St. Ag.	No.1	10	A	0	16.00	18.37	19.69	22.62	27.85	32.00	25.99	25.99,	
	No.2	10	В	0	21.11	24.25	27.85	32.00	36.75	39.39	32.00	29.85	
	No.3	, 10	С	0	9.84	11.31	12.12	14.92	16.00	13.92	11.31	9.18	
	No.4	10	D	0	14.50	16.00	18.37	21.11	17.14	17.14	16.00	13.10	

S.S: serum sample. Ch.g. chicken group. St.Ag. strain antigen.

Pre-v.: pre-vaccination.

Third Inter. Sci. Conf., 29 Jan.- 1 Feb./ 2009, Benha & Ras Sudr, Egypt

Fac. Vet. Med. (Moshtohor), Benha Univ

Table (3): Geometric mean of haemagglutination- inhibition (HI) antibody titers in sera of chicken vaccinated with different infectious coryga vaccines.

St. Ag.	vaccine	No. of s.s	Ch.	Weeks post-vaccination								
				Pre.v	2 .	4	6	. 8	10	12	14	16
W st. Ag.	No.1	10	A	û	16.00	16.00	17.14	·21.11	24.25	25.99	22.62	21.11
	No.2	10	В	0	21.11	22.62	27.85	32.00	36.75	34.29	32.00	32.00
	No.3	10	С	0	10.55	12.99	14.92	16.00	13.92	12.12	10.55	9.18
	No.4	10	· D	0	13.1	14.50	16.00	17.10	16.00	13.10	13.10	11.90
	No.1	10	A	0	14.50	16.00	17.14	19.69	22.62	22.62	19.69	18.37
Modesto. st . Ag.	No.2	10	В	0	18.37	21.11	22.62	27.85	32.00	34.29	29.85	27.85
	No.3	10	С	0	9.84	11.31	12.12	14.92	12.99	11.31	9.18	8.00
	No.4	10	D	0	11.90	11.90	14.50	16.00	14.50	14.50	13.10	10.80
0222 St. Ag.	No.1	10	A	0	14.50	16.00	16.00	18.37	21.11	22.62	18.37	17.14
	No.2	10	В	0	16.00	18.37	21.11	24.25	29.85	32.00	27.85	25.99
	No.3	10	С	0	8.00	9.18	11.31	12.99	11.31	10.55	8.57	8.00
	No.4	10	D	. 0	9.80	10.80	13.10	14.50	13.10	11.90	11.90	; 10.80

Third Inter. Sci. Conf., 29 Jan.- I Feb./ 2009, Benha & Ras Sudr, Egypt

Fac. Vet Med (Moshtohor), Benha Univ

مقارنة فعالية زيت المونتانيد • ٥ ، سابونين وكالسيوم فوسفات كمحسنات للقاح زكام الطيور · المعدى

أسامه السباعي كمال معهد بحوث الأمصال واللقاحات البيطرية – العباسية – القاهرة

# الملخص العربي

تم تحضير أربعة أنواع من لقاح زكام الطيور المعدي باستخدام زيت البرافين ، زيت المونتانيد و ، مسابونين وكالسيوم فوسفات كمحسنات للقاح . تم تقييم اللقاحات المحضرة في الكتاكرت عمر السبوع باستخدام اختبار التحدي والاختبارات السيرولوجية المختلفة. أثبتت النتائج إن لة ح زكام الطيور المعدي المحضر باستخدام زيت المونتانيد ، ٥ كمحسن أعطى اعلى استجابة مناعية ومعدلات حماية في الكتاكيت يليه اللقاح المحضر باستخدام زيت البرافين ثم كالسيوم أوسفات كمحسن بالترتيب . أوضحت النتائج أيضا إن استخدام سابونين كمحسن للقاح زكام الطيور المعدي أعطى أقل استجابة مناعية ومعدلات حماية في الكتاكيت بالمقارنة بالمحسنات الأخرى .