

IMPROVEMENT OF RABBIT PASTEURELLOSIS VACCINE USING MONTANIDE ISA50

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Abstract

Montanide ISA50 was used as an adjuvant for preparation of inactivated rabbit pasteurellosis vaccine, and compared with the commercially produced oil inactivated rabbit pasteurellosis vaccine adjuvanted with mineral oil. Two groups of rabbits were inoculated separately by first dose of these vaccines followed by second dose after 21 days from the first dose, the mineral oil used for first group and ISA50 used for the second group as adjuvant, meanwhile, the third group was left as non-vaccinated control group.

Cellular immunity was evaluated by using cell proliferation test and macrophage activity test (phagocytic percentage, phagocytic index). Humoral immune response was evaluated by using indirect haemagglutination test (IHA) and enzyme linked immunosorbent assay (ELISA). The results of the aforementioned tests proved that the two types of adjuvant induced protective titre against the diseases, but Montanide ISA50 oil adjuvant gave higher and longer duration of antibody level than the mineral oil. Challenge test and protection test in rabbit and mice gave similar results.

INTRODUCTION

Pasteurellosis due to *Pasteurella multocida* is an important disease in both commercial rabbit farm, as well as in laboratories using rabbits for research purposes. A variety of clinical signs and lesions have been associated with such *P. multocida* infection in the domestic rabbit. Snuffles due to rhinitis, chronic bronchopneumonia and acute fibrinous bronchopneumonia are patterns of diseases seen in the respiratory tract. Conjunctivitis, purulent otitis media, localized abscessation, genital tract infection and septicemia are all patterns of diseases associated with pasteurella infection. So commercial rabbitries are exposed to economic losses due to pasteurellosis (Mukkur *et al.*, 1982).

A variety of procedures have been used to reduce or eliminate pasteurellosis in rabbitries, including the establishment of pasteurella-free colonies, elimination of carrier by repeated culture of nasal swap sampling for *Pasteurella multocida* and vaccination with an inactivated vaccine (Mukkur *et al.*, 1982).

Mineral oil emulsions have been widely used to produce a prolonged slow release of antigen from aqueous phase. They have been used with antigen to potentiate the antibody response, also, to prolong their action (Cox and Coulter, 1997,

Aucoutuies *et al.*, 2001). Tween-80 is known to cause reduction in viscosity, interfacial tension in coagulation rate of aqueous globules of water in oil emulsion (Chiejina and Sowell, 1974).

Montanide ISA50 adjuvant is characterized by high effectiveness in generating strong immune response, easily manufactured with smooth, abscess free, stable and safe at storage (Seppic, 1994).

The purpose of this study was designed to use Montanide ISA50 adjuvant for preparing rabbit pasteurilla vaccine in a trial to improve immune response of rabbits to pasteurellosis in comparison to the ordinary mineral oil adjuvanted vaccine.

MATERIALS AND METHODS

A virulent local strain of *P. multocida* serotype A & D were obtained from Aerobic Bacterial Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These serotypes were used for production of commercial and our experimental *P. multocida* vaccine, serological tests as well as challenge test.

Laboratory animals

Healthy fifty rabbits with an average body weight of 2.5-2.5 kg were obtained from private farm and kept under strict hygienic measures. These rabbits were proved to be fully susceptible to the *P. multocida* by serological testing of all serum samples.

White mice with an average weight of 18 g were used for determination of the protective value of the two different adjuvanted vaccines according to Ose and Muenster (1968).

Adjuvants

1. Oil adjuvant ISA50: It was obtained from SEPPIC Cosmetics, Pharmacy Division, Paris, France. The ratio of adjuvant to the antigen (Volume / Volume) was done according to manufacturer instructions.

2. Mineral oil: The product of MICBIL, Alexandria, Whiterex 305, May 400 was used.

Emulsifiers

- Sorbitan monoleate (Span 80) was supplied by Ubichem LTD.
- Polyxythene sorbitan (Tween 80) was supplied by Sigma Company, USA.

P. multocida propagation, titration and inactivation

Each serotype of *P. multocida* A & D was propagated separately in trypticase Soya broth at 37°C aerobically for 24 hours to obtain a dense culture containing approximately 3.25×10^{10} cfu/ml of each strain. After 18-24 hours, inactivation was done by addition of 0.25 % formalin, each culture was tested for purity, safety and sterility as mentioned by Mukkur *et al.* (1982), then, equal amounts of culture were

mixed and preserved in 0.01 % of thiomersal and stored at 4°C until preparation of vaccines emulsion.

Preparation of inactivated *P. multocida* vaccines

1. Preparation of inactivated *P. multocida* vaccine using mineral oil It was prepared according to Stone *et al.* (1978).

2. Preparation of inactivated vaccine using Montanide ISA50

The vaccine was prepared by mixing 50 ml of *P. multocida* serotypes with 50 ml of Montanide ISA50 (volume/volume) according to manufacturer instructions.

Quality control of the vaccines

Quality control including purity, sterility and safety tests was carried out according to the Code of American Federal Regulation (1985).

Characterization of the vaccines

For evaluation of the emulsification process of the vaccines drop test, emulsion viscosity and emulsion stability were done according to Becher (1965).

Efficacy of the two prepared vaccines

The following tests were traced to verifying the efficacy of the two prepared vaccines:

1. Evaluation of cell mediated immune response

a. Cell proliferation test

Using proliferation kit with XTT reagent according to Scudiero *et al.* (1988).

b. Macrophage activity test

It was performed by the method of Barry *et al.* (1988), which was modified by El-Enbawy (1990).

Phagocytic percentage =

$$\frac{\text{No. of phagocytes which ingest candida}}{\text{Total no. of phagocyte}} \times 100$$

Phagocytic index =

$$\frac{\text{Total no. of phagocytes which ingest more than two candida}}{\text{Total no. of phagocyte which ingest candida}}$$

2. Evaluation of humoral immunity

It depends on several serological tests as follows:

a. Indirect haemagglutination test (IHA)

This test was conducted to determine the antibody titre against *P. multocida* as mentioned by Carter and Rappy (1962).

b. Enzyme Linked immunosorbent assay (ELISA)

It was done for determination of antibodies of *P. multocida* by the method of Briggs and Skeels (1984).

3. Challenge test

Fifty vaccinated and control rabbits were challenged with 0.1 ml of 100 LD₅₀ of virulent *P. multocida* serotypes A & D 15, days post-boostering. Observation period was 15 days post-challenge and any mortality was recorded.

4. Determination of log protection (in mice)

According to Ose and Muenster (1968).

Experimental Design

Rabbits were divided into 3 groups as shown in the following table:

Table 1. Experimental schedule.

Groups	Group (1) *	Group (2) **	Group (3) ***
1 st vaccinal dose (S/C)	1 ml	1 ml	Non
Blood collection	From the first week post-vaccination till 16 weeks		
2 nd vaccinal dose (booster dose) 3 weeks post 1 st dose S/C	1 ml	1 ml	Non
Challenge test	Challenge by using virulent strain of <i>P. multocida</i> serotypes S/C 15 days post-boostering		

* Group (1): Rabbit vaccinated with rabbit pasteurellosis vaccine adjuvanted with mineral oil

** Group (2): Rabbit vaccinated with rabbit pasteurellosis vaccine adjuvanted with Montanide ISA50

*** Group (3): Unvaccinated rabbits (Control)

RESULTS AND DISCUSSION

Pasteurellosis is one of the most common diseases in rabbits and results in heavy economic losses in rabbitries. Control of that disease is still as subjected of interest and usually attract the attention of researchers to know more and more about the disease epidemiology and how to control it in rabbits.

Vaccination is still considered as one of the major tools for controlling the disease. Vaccine efficacy depends on many variables, such as the nature, the amount of antigen administrated and the presence of adjuvants to enhance immunogenicity (Stone *et al.*, 1978).

Adjuvant is a substance that when added to a vaccine will enhance immunogenicity of an antigen, in the stimulation and evolution of humoral immune response. In some instances adjuvants also stimulate a cell mediated response by trapping antigen at sites where they are accessible to reactive lymphocytes, and they

induce antigen presenting cells to express costimulatory molecules such as CD₈₀. Also, adjuvant can reduce the quantity of antigen needed to generate a protective immune response and enables the vaccine to be cheaper, they can prolong the immune response of the vaccinated animals and birds (Stewart-Tull, 1994 and Aucoutuies *et al.*, 2001).

In this study, we compare between two types of adjuvants (Montanide ISA50 and mineral oil) for improving the immune response of rabbit against rabbit pasteurellosis.

The data given in Table 2 showed the cellular immune response of rabbit vaccinated with 2 different adjuvanted vaccines in which the phagocytic percentage and index gave high activity through the days. There was noticeable difference between vaccinated groups and control (non-vaccinated) group. The results agree with Amal *et al.* (2005, Elham and Mervat 2006). They found that the vaccine prepared with Montanide ISA50 oil adjuvant appeared to be more potent and gave protective level up to 6 months in comparison with the commercially used vaccine that contains mineral oil and gave protection up to 4 months only.

The results in Table 3 indicated that the lymphocytes proliferation revealed the gradual increase for different groups till 21 days post- vaccination, but there was a decrease in values in group I (vaccine adjuvanted with mineral oil) at 28 days.

Humoral immune response of *P. multocida* was determined by IHA test (Table 4) and ELISA (Table 5). These tests showed that the earlier, good immune response and higher antibodies against *P. multocida* occurred in second group of rabbits which received vaccination of *P. multocida* vaccine containing oil ISA50 than first group which received vaccination with the same vaccine adjuvanted with mineral oil from first week post- vaccination (WPV) till the end of experiment. These results agreed with William and Johon (1989), Seppic (1994), Abdel Wanis *et al.* (2003), Amal *et al.* (2005) and Elham and Mervat (2006).

The results of challenge test against *P. multocida* tabulated in Table 6 proved that Montanide ISA50 adjuvanted vaccine gave protection 100% percent and 90% against serotypes A & D, respectively, while, mineral oil gave 80% and 80% protection against serotypes A & D, respectively. These results were in agreement with Amal *et al.* (2005), Elham and Mervat (2006).

Also, the protective value of the 2 different adjuvant vaccines was determined by the log protection in mice which showed that the log protection of Montanide ISA50 adjuvanted vaccine was 3.85 and 3.69 for types A & D, respectively, while, the log protection of mineral oil adjuvanted vaccine was 3.15 and 2.98 for types A & D, respectively (Table 7).

So, the results of the present study concluded that both types of adjuvanted vaccines induced a considerable immunity in rabbits, but, Montanide ISA50 gave higher and long duration of antibodies response and protection in addition to its preparation with an easier technique.

Table 2. Evaluation of cell mediated immune response in different rabbits of vaccinated groups by macrophage activity using candida albicans expressed by phagocytic percentage and phagocytic index.

Groups of rabbits	Phagocytic percentage			Phagocytic index		
	Days post vaccination			Days post vaccination		
	7	14	21	7	14	21
1	77.78	84.62	58.33	0.86	0.82	0.71
2	71.43	89.46	87.50	0.80	0.86	0.79
3	13.90	14.10	13.10	0.09	0.06	0.06

Group (1): Rabbits vaccinated with rabbit pasteurellosis vaccine adjuvanted with mineral oil.

Group (2): Rabbits vaccinated with rabbit pasteurellosis vaccine adjuvanted with Montanide ISA50.

Group (3): Non-vaccinated rabbits (Control).

Table 3. Lymphocytic proliferation test for vaccinated rabbit groups expressed by optical density.

Groups of rabbits	Optical Density			
	Days post vaccination			
	7	15	21	28
1	0.458	0.460	1.08	0.547
2	0.425	0.350	0.848	0.903
3	0.112	0.097	0.125	0.123

Group (1): Rabbits vaccinated with rabbit pasteurellosis vaccine adjuvanted with mineral oil.

Group (2): Rabbits vaccinated with rabbit pasteurellosis vaccine adjuvanted with Montanide ISA50.

Group (3): Non-vaccinated rabbits (Control).

Table 4. Comparative results of anti-*Pasteurella multocida* antibodies in sera of rabbits vaccinated with *P. multocida* vaccine using different adjuvants measured by IHA test.

Type of adjuvanted vaccine	Type of antigen	Pre-vaccination	Weeks post vaccination									
			1	2	3	4	6	8	10	12	14	16
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with mineral oil	A	8	52	160	510	780	1650	1730	1580	1390	985	598
	D	7	49	140	500	720	1590	1750	1520	1310	940	509
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with Montanide ISA50	A	8	92	298	988	1385	2350	2490	2385	2240	1610	1120
	D	8	88	289	890	1250	2290	2418	2370	2205	1590	1080
Control unvaccinated group	A	8	8	9	9	8	9	10	10	9	7	8
	D	7	8	9	8	9	8	9	10	9	8	8

Table 5. Comparative results of anti-Pasteurella multocida antibodies in sera of rabbits vaccinated with *P. multocida* vaccine using different adjuvants measured by ELISA.

Type of adjuvanted vaccine	Type of antigen	Pre-vaccination	Weeks post vaccination									
			1	2	3	4	6	8	10	12	14	16
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with mineral oil	A	85	180	540	2180	3120	4250	4420	3150	3010	2030	1730
	D	87	140	520	2120	3109	4210	4480	3170	3050	2005	1680
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with Montanide ISA50	A	87	290	950	3750	4490	6530	4930	5620	5480	4570	3250
	D	86	280	910	3650	4485	6420	4900	5590	5350	4320	3035
Control unvaccinated group	A	85	88	90	95	94	95	92	93	90	88	89
	D	87	90	90	93	93	90	90	88	87	89	88

Table 6. Results of challenge test against virulent *Pasteurella multocida* 6 weeks post-first vaccination.

Type of adjuvanted vaccine	Challenge antigen	No. of rabbits	No. of <i>P. multocida</i> specific mortality	No. of mortality / Total	Protection %
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with mineral oil	A	10	2	2/10	80 %
	D	10	2	2/10	80 %
Rabbits vaccinated with <i>P. multocida</i> vaccine adjuvanted with Montanide ISA50	A	10	1	1/10	90 %
	D	10	0	0/10	100 %
Control unvaccinated group	A	5	5	5/5	0 %
	D	5	5	5/5	0 %

Table 7. Protective value of rabbit pasteurellosis vaccine using different adjuvants estimated in white mice*.

Challenge serotypes	Mice immunized with <i>P. multocida</i> vaccine adjuvanted with the following adjuvants	
	Mineral oil adjuvant	Montanide ISA50 adjuvant
A	3.15 **	3.85 **
D	2.98 **	3.69 **

* Protective value in logs protection

by Ose and Muenster (1968).

** Protective value ≥ 2 log

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تطوير لقاح التسمم الدموى الأرنبى باستخدام المونتانيدي أس50

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فى هذه الدراسة تم استخدام زيت المانتونيد أس إيه 50 لتحضير لقاح التسمم الدموى الأرنبى لمقارنته مع نفس اللقاح المحضر محلياً والذي استخدم فيه الزيت المعدنى كمحفز مناعى، حيث استخدمت ثلاث مجموعات من الأرناب، المجموعتان الأولى والثانية تم تحصينهما بجرعتين من اللقاح بينهما 21 يوماً. وفى المجموعة الأولى تم استخدام الزيت المعدنى كمحفز مناعى للقاح، بينما فى المجموعة الثانية تم استخدام زيت المانتونيد أس إيه 50 كمحفز مناعى للقاح، أما المجموعة الثالثة فقد تركت كمجموعة ضابطة (كنترول) أى غير محصنة تماماً.

وقد أثبتت التجارب السيرولوجية المختلفة لقياس مستوى المناعة الخلوية والمناعية الخلطية بأن كلا اللقاحين المستخدمين أعطى كفاءة مناعية عالية مقارنة بالمجموعة الضابطة ولكن اللقاح الذى استخدم فيه المانتونيد أس إيه 50 كمحفز مناعى أعطى أعلى مستوى مناعى من الأجسام المضادة وأطول فترة مناعية مقارنة باللقاح الذى احتوى الزيت المعدنى كمحفز مناعى وقد تم تأكيد هذه النتائج بإجراء اختبار التحدى الذى أثبت نفس النتائج تقريباً. وعلى ذلك يمكن القول ان استخدام المانتونيد أس إيه 50 كمحفز مناعى يعطى أفضل كفاءة مناعية فى الأرناب.