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## TRIAL FOR ENHANCEMENT OF PROTECTION AGAINST SNUFFLES IN RABBITS

(With 2 Tables)

By

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محاولة لزيادة المقاومة ضد مرض الخنة في الأرانب

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تم إختبار التأثير المحفز للمناعة لمادة الأميونودينمك - 1 (ID-1) ضد العدوى بميكروب الباستيريللا ملتوسيدا في الأرانب . في هذه الدراسة تم تحصين الأرانب بواسطة لقاح الباستيريللا ملتوسيدا الفورماليني واللقاح الزيتي في مجموعات مختلفة من الأرانب من حقن مادة ID-1 في برامج مختلفة. تم تحديد المناعة المكتسبة نتيجة حقن اللقاح مع مادة ID-1 بواسطة قياس الأجسام المضادة في الدم بإختبار تلازن الدم السلبي وكذلك بإجراء إختبار تحدي المناعة. وقد أوضحت النتائج أن أعلى معدل للمناعة كانت في المجموعات المحصنة والمحقونة بمادو ID-1 في نفس الوقت.

### SUMMARY

The suspected immunopotentiator effect of ID-1 on the immune response and resistance to *Pasteurella multocida* infection was tested in rabbits. Different schemes of vaccination with *Pasteurella multocida* formalized and oil adjuvant vaccines were used in different groups of rabbits. High anti *Pasteurella multocida* antibodies and greater resistance to challenge infection with 100 LD50 of virulent *Pasteurella multocida* organism in the group of rabbits that received ID-1 and *Pasteurella multocida* vaccine simultaneously documented better immune response.

**Key words:** Trial for enhancement of Protection against Snuffles In Rabbits

## INTRODUCTION

*Pasteurella multocida* infection causes serious economic losses in breeding and raising rabbits. Rabbit pasteurellosis is endemic disease in most rabbitries. High husbandry standards, elimination of NH<sub>3</sub> and stabilization of the environment reduce the development of the more severe forms of the disease in adults (Harkness and Wagner, 1989). Snuffles, a chronic mucopurulent rhinitis and sinusitis are enzootic unless special precautions are taken to prevent it (Flatt, 1974). Several immunization programs have been tried in Egypt with varying degrees of success Zaher *et al.* (1976), where protective vaccines against pasteurellosis in controlled studies may not be effective under field conditions and stressed animals. Modulation of the immune response to *Pasteurella multocida* vaccine was an important factor in inducing protective immunity in vaccinated rabbits (Glass and Beastly, 1989).

Immunopotential means enhancement of the immune response that can be affected by increasing the rate at which the response occurs, elevating its magnitude, prolonging the response, or directing the response to a particular fact of the immune response (Hyde and Patnode, 1987).

The commercial use of microbial and nonmicrobial immunostimulants were now widespread all over the world. Several immunostimulants had been used in inducing cytokine production and hence increase resistance to infectious diseases (Mayer mediators in colostrum which act as immunopotentiators like interleukins and interferons as well as macrophages activating factors (Shalaby *et al.*, 1994; Khafagy, 1981). This remarkable defense mechanism may be due to the presence of the soluble mediator in colostrum which act as immunopotentiators like interleukins and interferons as well as macrophages activating factors (Shalaby, 1994 and khafagy *et al.*, 1994).

Poli (1984) had categorized immunopotentiators as biological and chemical products. This classification was further broken down by Mulcaly and Quinn (1986), so that three categories were defined namely, physiological products, Substances of microbial origin and synthetic compounds.

It was felt that this work design would provide insight into humoral capabilities of the rabbits towards the immunopotentiating agent studied. It was hypothesized that a more effective immunization



program against Snuffles disease could be achieved in rabbits with formalized and oil adjuvant vaccines, provided a component of the colostral milking of the bovine (LD-1) was employed which would induce antibody response.

## **MATERIALS and METHODS**

### **Experimental rabbits:**

Thirty-five Boscat rabbits weighing 1.5-2Kg of 2-3 month age were used in this study. They neither have the history of *Pasteurella multocida* infection nor vaccination. Blood samples were taken before vaccination was negative for *Pasteurella multocida* antibodies.

### **Immunodynamic -1 (I D-1):**

ID-1 derived from components of the first colostral milking of the bovine, it was composed of specific antibody protein and nonspecific protein which trigger the body defense system in animals to respond to diseases, obtained by Immunodynamic-Perry : Iowa. U.S.A.

### **Pasteurella multocida vaccines:**

Formalized and oil adjuvant *Pasteurella multocida* vaccines were obtained from Veterinary Serum and Vaccine Research Institute Abbasia, Cairo, Egypt.

### **Blood samples:**

For serological examination, blood samples were collected from different groups of rabbits before and after vaccination with different programs and serum samples were collected and stored at -20°C until being used.

### **Passive haemagglutination test:**

Passive haemagglutination test was carried out according to (Carter and Rappy 1962), for measuring antibodies against *Pasteurella multocida* in collected serum of rabbits.

### **Challenge test:**

All groups of vaccinated and control rabbits were challenged after 58 days post first vaccination with 100 LD50 of virulent *Pasteurella multocida* organisms. This dose was considered double the challenging dose commonly used to measure the immunity of vaccinated rabbits (Nagy and Penn 1975) in order to differentiate between the acquired immunity among the different groups of vaccinated rabbits.

### **Experimental design:**

Experimental rabbits were divided into five equal groups, each of seven animals.

**Group I:** Nonvaccinated and injected with ID-1

**Group II:** Vaccinated with *Pasteurella multocida* alone (first with formalinized and then after two weeks, with oil adjuvant vaccine).

**Group III:** ID-1 treated and after two weeks vaccinated with *Pasteurella multocida* formalized vaccine followed by second dose after another two weeks

**Group IV:** ID-1 treated and *Pasteurella multocida* formalized vaccine simultaneously and after two weeks with oil adjuvant vaccine.

**Group V:** Control untreated group (non vaccinated and non injected with ID-1).

## RESULTS and DISCUSSION

The immunopotentiating effect of ID-1 was scored by its marked hyperglobulinaemia manifested by very high significant  $P < 0.01$  increase of B, and  $\alpha$  globulin fractions in sera of chickens (Shalaby *et al.*, 1994), similar potentiating effect with vitamin E was recorded by Sheffy and Schultz (1979) who stated that vitamin E have been shown to have immunomodulatory effect in a variety of species, when administered in quantities excess of established dietary requirement.

Armanious *et al.* (1993) indicated the effective adjuvanticity of heat killed *Mycobacterium intracellulare*, Pind Avi and Ultracorn in enhancing the immunizing capacities of the CU strain of *Pasteurella multocida* vaccine in vivo and possibly to augment nonspecific resistance in chicken to *Pasteurella multocida* infection.

Table 1 results of the anti*Pasteurella multocida* antibodies in sera of differently vaccinated groups of rabbits. The highest titers were recorded in the group of rabbits simultaneously ID-1 treated and *Pasteurella multocida* formalized vaccine and after two weeks with oil-adjuvant vaccine, especially in the period from first week where it registered a significant high titer ( $P < 0.001$ ) 2560 till the fourth week post vaccination where it scored mean titer 1280. Also enhancement in antibodies mean titer was noted in the sera of rabbits primed with ID-1 treated then vaccinated with two of formalized *Pasteurella multocida*, where it revealed a highly significant ( $P < 0.001$ ) of mean titer of 2560 at the third week post vaccination, then declined suddenly at the fourth week to give a mean titer of 640. In comparison, with the group of rabbits vaccinated with both formalized then oil adjuvant *Pasteurella multocida* vaccine, it showed a slightly significance  $P < 0.01$  of mean titer 640 in the second and third week post vaccination, then decreased to 320



in the fourth week post vaccination. The control untreated group revealed antibody titers similar to that obtained with ID-1 treated group and no significant differences were noted between them.

Challenging results are shown in Table (2) using 100 LD50 *Pasteurella multocida* organisms (Nagy and Penn, 1975), to evaluate immune response to vaccination using different treatments. The best protection results were recorded in rabbits of group (4), simultaneously treated with ID-1 and *Pasteurella multocida* formalized and oil adjuvant vaccines. The protection rate was 71.4%, in comparison with 43% in both groups vaccinated with *Pasteurella multocida* formalized and oil adjuvant vaccines alone (group 2) and ID-1 treated then *Pasteurella multocida* formalized vaccine two weeks later (group 3). Although ID-1 treated group showed immune response, challenged rabbits of this group (1) and group (5) showed no protection

It could be concluded that ID-1 augmented the effect of *Pasteurella multocida* vaccines in rabbits. This was observed in better protection among rabbits especially those were simultaneously vaccinated with *Pasteurella multocida* vaccine and ID-1 treated group, than other rabbits vaccinated with *Pasteurella multocida* vaccines alone.

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Table (1): Seroconversion of *Pasteurella multocida* antibodies as measured by indirect haemagglutination test in groups illustrated.

Group	Number of rabbits/ group	Treatment	Haemagglutination antibody titres				
			Pretreatment	3 weeks post treatment	Post second dose of vaccination		
				1 <sup>st</sup> w	2 <sup>nd</sup> w	3 <sup>rd</sup> w	4 <sup>th</sup> w
1	7	ID-1 treated group	20	80	80	40	40
2	7	<i>P. multocida</i> vaccinated group	20	320	320	640	320
3	7	ID-1 previous to <i>P. multocida</i> vaccination	20	160	160	1280	640
4	7	ID-1 and <i>P. multocida</i> vaccination simultaneously	20	1280	2560	2560	1280
5	7	Control untreated group	20	40	40	40	40

Table (2): Protection pattern of rabbit vaccinated against *P. multocida* post challenge with (2 x) 100 LD<sub>50</sub> of virulent strain of *pasteurella multocida*.

Group	Number of rabbits	Treatment	Most altry pattern				Total mortality	Survival ratio	Protection %
			24 hr	48 hr	72 hr	96 hr			
1	7	ID-1 treated group	5	2	-	-	7/7	0/7	-
2	7	<i>P. multocida</i> vaccinated group	-	1	2	1	4/7	3/7	43%
3	7	ID-1 previous to <i>P. multocida</i> vaccination	1	2	1	-	4/7	3/7	43%
4	7	ID-1 and <i>P. multocida</i> vaccination simultaneously	-	1	1	-	2/7	5/7	71.4%
5	7	Control untreated group	7	-	-	-	7/7	0/7	-