

Effect of propionobacterium and E.Coli lipopolysaccharide (immunair 17.5) immunomodulator on response of rabbits to RHDV vaccine

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The present study was conducted to study the immunomodulatory effect of combined of extract of propionobacterium and E.coli lipopolysaccharide (immunair 17.5) to enhance the immune response of rabbits to rabbit haemorrhagic disease virus (RHDV) vaccine. Forty New Zealand rabbits aged 2 months with average weight 1.5-2 kgs were divided into 4 equal groups. Group (1) was vaccinated with RHDV vaccine and the immunomodulator, group (2) was only vaccinated with RHDV vaccine, group (3) was received the immunomodulator only and the last group was kept as non-vaccinated, no-treated control. The results revealed that three days oral administration of the immunomodulator under test at time of RHDV vaccination had an improving effect on both humoral and cell mediated immune response of rabbits to RHDV vaccine. Results obtained by challenge test come in harmony with serological test.

Rabbit haemorrhagic disease (RHD) is a contagious highly fatal disease of rabbits, characterized by a high morbidity and mortality (Capucci *et al.*, 1997).

The common features of the disease are anorexia, dyspnoea, abdominal respiration, abortion in pregnant does, bloody nasal discharge, organs excitement and convulsion followed by rapid collapse and death (CAP, 1989). The characteristic pathological lesions were haemorrhages in respiratory system, liver, spleen, cardiac muscles and occasionally in the kidney (Parra and Prieto, 1990). Due to viral etiology of the disease, vaccination is considered the efficient method to eradicate the disease (Daoud *et al.*, 1998). Rabbits may be exposed to immunosuppressive factors, such as concomitant infection with myxomatosis, pasteurellosis or coccidiosis (Gergis *et al.*, 1993) as well as physiological factors.

Another reason for vaccination failure under field conditions could be due to management errors, bad hygiene and uncontrolled environment.

Immunomodulators can play a very important role in enhancing the response of the immune system to a particular antigenic stimuli as brought by the process of vaccination (Gatenby, 1998), or by restoring the immunodepressive effect of various factors (Chedid *et al.*, 1986).

The objective of this work was studying the possible immunopotentiating effect of a biological substance prepared from inactivated cells of *Propionobacterium acnes* and lipopolysaccharide from *Escherichia coli* on both the humoral and cellular immune response of rabbits vaccinated with the inactivated RHD vaccine.

Material and methods

Experimental animals. Forty white New Zealand rabbits (aged 2 months with average weight 1.5-2 kgs) were purchased from private rabbiteries which had neither a history of RHDV infection nor vaccination. They were seronegative for RHD virus antibodies.

Virus. Virulent RHDV (local isolate) identified against reference immune serum by Prof. Dr. H.M.Hafez, Free Univ., Berlin, Germany during M.V.Sc. thesis (Poultry & Rabbit Diseases) by (Salman, 1999). It was sequenced and submitted to GenBank with accession No. EF488823, (Salman, 2007). It had a titre of 2048 HA unit/ml and LD₅₀ of 10^{6.5}/ml.

The commercial Immunomodulator (immunair 17.5). Each 1 ml of the immunostimulant (Laboratorios Calier, S.A. Spain) contained inactivated *propionobacterium acnes* cells (0.17 mg) and lipopolysaccharide from apathogenic *E.coli* cell wall (0.05 mg). Administration was in the drinking water at the range of 1 ml/10 Kg

body weight, every 24 hours during 3 consecutive days.

Preparation of RHDV vaccine. The vaccine was prepared according to (Arguello Villares, 1991; Salman, 1999), where livers of dead experimentally infected rabbits with RHDV, were homogenized in phosphate buffered saline (pH 7.2) and was centrifuged at 3000 rpm (15 minutes) at 4 °C. The supernatant was collected and inactivated with 0.4% formalin at 37°C/48 hours. After safety examination, the vaccine was ready for use in experimental rabbits. The suspension was adjuvanted with aluminum hydroxide gel (Honel, UK) at a final concentration of 20%. The recommended vaccine dose (0.5 ml/animal) contained protective virus titre (1024 HA unit), was inoculated S/C as stated by (Smid *et al.*, 1991).

Vaccination. Rabbits were divided into 4 groups (10 rabbits/group), group (1) was vaccinated S/C with RHDV vaccine (0.5 ml/animal) in combination with immunair 17.5 in drinking water. Group (2) was only vaccinated with RHDV vaccine. Group (3) was received immunair 17.5 only in drinking water. The last group was kept as non-treated-non-vaccinated controls. Blood samples were collected at regular intervals for evaluation of immune response for 24 weeks.

Evaluation of humoral immune response.

Haemagglutination inhibition test (HI). It was performed according to the method previously described by (Pu *et al.*, 1985).

ELISA test. The test was carried out according to (Smid *et al.*, 1991).

Evaluation of the cell mediated immune response.

Lymphocytes blastogenesis assay. It was carried out by tetrazolium calorimetric assay according to (Mosmann, 1983) and the results were expressed as delta optical density (ΔOD).

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

Phagocytic % =

$$\frac{\text{No of phagocytic cells which ingest candida}}{\text{total No of phagocytes}} \times 100$$

Phagocytic index. It was done according to Richardson and Smith (1981).

Phagocytic index =

$$\frac{\text{Total No of phagocytes which ingest more than two candida}}{\text{Total No of phagocytes which ingest candida}}$$

Potency test. All rabbits were challenged with 100 LD₅₀ of virulent fully characterized RHDV (Giza 1997) after three weeks post

vaccination, and observed for signs of RHDV for one week after challenge infection (Salman, 2007).

Results and Discussion

Interest in the topics of immunomodulation has expanded rapidly in recent years, with the realization of its potential in human and veterinary medicine (Barlitt and Kreider, 1981). Recently, non-specific immunostimulant are gaining increasing attention in recent years to counteract the effect OF environmental immunosuppressive factors and help the animal in its struggle against disease causing agents and potentiating of its immune response to applied vaccines.

This work was directed to demonstrate the possible promoting effects of the incorporated immunomodulator prepared from extracts of propionobacterium and lipopolysaccharides of *E.coli* on the immunogenic properties of RHDV vaccine given to rabbits.

Humoral immune responses against RHD virus was estimated by HI and ELISA test as shown in tables (1 and 2), respectively. The results revealed that the antibody titers in rabbits received immunair 17.5 immunomodulator with RHDV vaccine (G1) began earlier and higher than those vaccinated only (G2) and non-treated.

These results accord with those of Tizzard (2000) who stated that propionobacterium acnes, posses adjuvant activity which enhance antibody formation against viral infection.

Concerning the cellular immune response, results of lymphocyte blastogenesis as represented in table (3) revealed that a maximum response of T cells expressed as ΔOD in animals of group 1 (vaccinated and treated with the immunomodulator) in comparison with those vaccinated with RHD vaccine only (G2) .

More confirmation of cellular immune response was also achieved using macrophage activity test (tables 4 and 5) as expressed by phagocytic index and phagocytic percentage respectively were running parallel to the former test.

The recorded improvement in the measured immune parameters can be attributed to the LPS contains of the used immunomodulator as mentioned before by (Johnson 1975; Waksman, 1979) has recording increase the phagocytic activity and the production of inflammatory cells, they also stimulate the production of interferon and activate complement.

Schultze and Goodman (1979) stated that the immunomodulation effect is manifested by the

Table (1): Mean haemagglutination inhibition titers (log₂) induced by RHDV vaccine.

Group	Treatment		Weeks post vaccination						
	Vaccine	I.M.*	2	4	8	12	16	20	24
G 1	+	+	8.5	8.8	8.7	9.6	9.7	9.2	7.9
G 2	+	-	7.6	8.7	8.8	8.3	8	7.8	6.3
G 3	-	+	0	0	0	0	0	0	0
G 4	-	-	0	0	0	0	0	0	0

*I. M. Immunodulator.

Table (2): ELISA mean optical density in sera of rabbits vaccinated with RHDV vaccine (mean absorbance values).

Group	Weeks post vaccination						
	2	4	8	12	16	20	24
G 1	0.05	0.72	0.952	1.11	0.96	0.93	0.89
G 2	0.5	0.71	0.89	0.80	0.76	0.73	0.70
G 3	0.06	0.09	0.05	0.15	0.05	0.05	0.04
G 4	0.058	0.49	0.05	0.05	0.05	0.06	0.06

Table (3): Evaluation of cell mediated immune response by MTT of treated rabbits.

Groups	Days post vaccination			
	4	11	18	25
G1	0.093	0.083	0.104	0.010
G2	0.035	0.044	0.109	0.005
G3	0.024	0.0285	0.010	0.001
G4	0.004	0.012	0.011	0.010

Table (4): Phagocytic activity of rabbits vaccinated by RHDV vaccine and treated with immunair 17.5 expressed by phagocytic index.

Groups	Weeks post vaccination		
	1	2	3
G1	0.77	0.857	0.55
G2	0.75	0.810	0.50
G3	0.22	0.260	0.12
G4	0.10	0.090	0.08

Table (5): Phagocytic activity of rabbit vaccinated by RHDV vaccine and treated by immunair 17.5 expressed by phagocytic percentages.

Groups	Weeks post vaccination		
	1	2	3
G1	74.20	77.7	70.00
G2	70.00	72.72	69.20
G3	25.60	15.57	9.400
G4	11.11	10.50	10.00

Table (6): Potency test of immunized rabbits challenged by 100 LD₅₀ RHDV.

Group	No. of rabbits	Died/survived	Total survived number	Protection %
G1	10	0/10	10	100 %
G2	10	1/9	9	90 %
G3	10	8/2	2	20 %
G4	10	10/0	0	0

synthesis and secretion of immunoglobulins enhancing the humoral immune response.

Propionibacterium acnes have been reported to stimulate non-specific resistance to viral infection (Carter and Wagner, 1984) activate both

B and T lymphocytes, as well as activate to macrophages (Frost and Lance, 1973).

Results of challenge test (table 6) revealed that rabbits vaccinated with RHDV vaccine and treated with the immunomodulator (G 1) gave

high protection (100%) compared to 90% in rabbit received RHDV vaccine only (G 2) and 20% in treated group (G 3). The bioassay test against RHDV vaccine confirmed the above mentioned humoral and cell mediated immune response.

In conclusion, the orally administration of immunomodulator in combination with RHDV vaccine appear to show a tendency in putting off the pathogenesis of RHDV , and it can be of value in reducing the incidence of such viral infection if used under rabbiteries conditions in Egypt.

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تأثير المحفز المناعي مستخلص البروبيونوباكتريم والليبوبولي سكاريد لميكروب الإيشريشيا كولاي (إميونير ١٧,٥) على الإستجابة المناعية للأرناب المحصنة بلقاح النزف الدموي الفيروسي الأرنبي

صممت هذه التجربة لدراسة تأثير المحفز المناعي والمكون من مستخلص البروبيونوباكتريم والليبوبولي سكاريد لميكروب الإيشريشيا كولاي لزيادة الإستجابة المناعية للقاح النزف الدموي الفيروسي الأرنبي. تم إستخدام أربعون أرنب (أعمارها شهرين ومتوسط أوزانها ١,٥ - ٢ كجم) تم تقسيمها إلى أربعة مجموعات متساوية. المجموعة الأولى تم تحصينها بلقاح النزف الدموي مع معالجتها بالمحفز المناعي و المجموعة الثانية تم تحصينها بلقاح فقط والمجموعة الثالثة تم معالجتها بالمحفز المناعي فقط. أما المجموعة الأخيرة فتم إستخدامها كضوابط سلبية للتجربة. أظهرت النتائج أن إستخدام المحفز المناعي إميونير ١٧,٥ مع التحصين بلقاح النزف الدموي قد أدى إلى تحفيز مناعي عالي ظهر من خلال القياسات المناعية سواء المصلية أو الخلوية عنها في المجموعات الأخرى و كانت نتائج إختبار تحدي المناعة بإستخدام العترة الضارية للفيروس متوافقة مع النتائج المناعية و الخلوية.

