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CELLULAR AND HUMORAL IMMUNE RESPONSES IN LAMBS TREATED WITH BCG OR ULTRA-CORN

(With 4 Tables)

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الاستجابة المناعية للحملان المعالجة بالبى سى جى أو الالتراكورن

عمر سلامة ، بدير عجاج ، عزت عفيفى ، نعيمة عفيفى ، ابراهيم الصعيدى

استهدفت هذه الدراسة تقييم الاستجابة المناعية للحملان الرحمانى المعالجة بالبى سى جى او الالتراكورن وقد اجريت هذه الدراسة على ٤٨ حمل رحمانى عمر ثلاثة شهور بمزرعة السيرو التابعة لمعهد بحوث الأنتاج الحيوانى حيث قسمت الى ثلاث مجموعات عولجت المجموعة الأولى بالبى سى جى والثانية بالالتراكورن وتركت الثالثة كمجموعة ضابطة. تم تقدير العد النوعى لكرات الدم البيضاء و كل من الجلوبيولينات المناعية الكلية , الجلوبيولينات المناعية-ج ,الجاما جلوبيولين بالمصل لمدة أربعة أسابيع بعد العلاج كما تم قياس حساسية الجلد للتيوبر كلين بعد ٦ شهور من العلاج. وقد اوضحت النتائج زيادة معنوية فى الليمفوسيت و المونوسيت وزيادة فى سمك الجلد فى المجموعات المعالجة بالمقارنة بالمجموعة الضابطة وكانت هذه الزيادة اكثر وضوحا فى المجموعة المعالجة بالبى سى جى كما اوضحت النتائج زيادة قيم الجلوبيولينات المناعية الكلية ، الجلوبيولينات المناعية ج فى كلا المجموعتين المعالجة بالبى سى جى والمعالجة بالالتراكورن.

SUMMARY

In this investigation, the BCG and Ultra-corn were evaluated for their effect on cellular and humeral immune responses in Rahmani lambs. The results obtained revealed that the BCG vaccinated lambs have an efficiently stimulated cell mediated or delayed hypersensitivity (DTH) than those

vaccinated with Ultra-corn. These lambs showed also a sharp increase in the lymphocyte and monocyte count at 7, 14, 21 and 28 days post-vaccination. On the other hand, both vaccines stimulated moderate increase of total immunoglobulins (Igs) and immunoglobulin-G (IgG) and consequently gamma-globulin, 14, 21 and 28 days post-vaccination.

Key words: *Lambs - BCG - Immune Responce.*

INTRODUCTION

Several authors demonstrated that the application of nonspecific immunostimulants such as BCG and Ultra-corn are capable of rising resistance of cattle, sheep and goats against viral, bacterial and parasitic diseases (Barakat, 1979; Barakat et al., 1981; Awad et al., 1982; El-Balkemy et al., 1983 and Shalaby et al., 1992). BCG acts as an immunostimulant, it has both specific and nonspecific actions (Fudenberg et al., 1977). Nonspecifically it protects against all microbes that can live intracellularly such as Brucella abortus, Tubercle bacillus, salmonella enteritidis, listeria monocytogenes, staphylococcus aureus, Streptococcus pyogenes, viruses, prototozoa, parasites and fungi (Barakat, 1979). Corynebacterium cutis when injected into the animal are phagocytosed by macrophages and stimulate the release of tumor necrosis factor-and interleukin. These monokines may have a secondary effect on lymphocyte function and so stimulate the immune system nonspecifically (Shalaby et al., 1992).

It is observed that the susceptibility of lambs to intestinal and respiratory infections is being increased during the period of weaning, Hence the objectives of this work were to elucidate the effect of BCG or Ultra-corn vaccination on cellular and humeral immunity in lambs before weaning.

MATERIAL and METHODS

BCG vaccine: A live-freeze dried vaccine from an attenuated strain of mycobacterium bovis was used for vaccination of lambs. It is a product of Pasteur Marieux Lyone (France). When reconstituted with its diluent, 1 ml contains 10 doses for children over year of age. It was obtained from Egyptian Organization for Biological vaccine production, Cairo, Egypt.

ULTRA-CORN vaccine: Ultra-corn is an ultrasonically lysated suspension of coryne bacterium cutis in a concentration of 20 mg/ml. It is manufactured by Virbac Laboratories (France).

Tuberculin: The purified protein derivative (Mammalian PPD Tuberculin) used as single intradermal tuberculin test was obtained from Serum and Vaccine Research Institute, Cairo, Egypt.

Skin test: Six months after BCG or Ultra-corn inoculation, skin reaction of vaccinated and nonvaccinated lambs were tested by measuring the difference in double skin fold thickness before and 72 hours after intradermal inoculation of 0.1 ml tuberculin (10000 TU).

Animals: Forty eight Rahmani lambs about 3 months of age were divided into 3 groups, 16 lambs of each as follows:

Group I : Vaccinated intradermally with 0.1ml BCG.

Was divided into 2 subgroups, 8 lambs of each. Subgroup A : Vaccinated intramuscularly with 20 mg Ultra-corn. While, subgroup B were vaccinated with 40 mg Ultra-corn .

Group III: Were kept as nonvaccinated control.

Lambs were weighed on zero day and then every 2 weeks for 5 months and kept under close observations. The symptoms of diseases and their duration in the different groups of lambs were also recorded.

Blood samples were obtained from lambs at 7, 14 and 28 days post-vaccination and used to measure differential leucocytic count, serum total proteins, protein electrophoretic pattern, total immunoglobulins (Igs) and immunoglobulin-G (IgG). Differential leucocytic count was calculated after Schalm et al., (1975) .Total proteins in serum samples were determined by the method given by Sonnenwirth and Jarett (1980), and fractionated after Davis (1964) and Ornstein (1964). Serum Igs were determined by sodium sulphite turbidity test according to Stone and Gitter (1969) by using spectrophotometer for quantitation as described by Khalil (1975). Quantitation of serum IgG was done by radial immunodiffusion using specific kits obtained from the Binding site limited, London, England.

RESULTS

Statistical analysis of the results for cellular and humoral immune responses are presented in tables (1, 2, 3 and 4). Table (1) showed a significant increase in the lymphocytes in BCG (group I) and Ultra-corn (subgroup 2 B) vaccinated lambs all over the periods of the experiment and a significant increase in the monocytes in group I at 21 and 28 days post-vaccination. A noticeable alteration in the neutrophil/ lymphocyte ratio has occurred in vaccinated lambs as compared with non-vaccinated ones. The

difference in lymphocyte count was remarkable in BCG vaccinated lambs but was slight in Ultra-corn vaccinated lambs.

It is indicated from Table (2) that BCG vaccinated lambs responded to vaccination as they were converted from tuberculin negative to tuberculin positive after being tested with single intradermal tuberculin test, 6 months post-vaccination. This response was not quite clear in Ultra-corn vaccinated lambs.

Picture of fractionation of serum proteins revealed significant differences only in gamma-globulin bands (Table 3). Total gamma-globulin showed an elevated values at 14 and 21 days post-vaccination in group I and group II B, respectively- Gamma-1 and Gamma -2 and consequently total gamma-globulin are significantly increased at 28 days post-vaccination in both groups.

From Table 4, it is clear that Igs and IgG levels in the sera of vaccinated lambs reached their maximum levels 3 weeks post-vaccination. In group I the IgG increased significantly 2 weeks post-vaccination and continued up to 4 weeks post-vaccination. In group II, the IgG values showed a significant increase only in lambs inoculated with 40 mg Ultra-corn (Subgroup II B), 3 and 4 weeks post-vaccination. serum total proteins recorded a significant elevated values in group I and subgroup II B, 4 weeks post-vaccination.

Among BCG vaccinated lambs, 2 lambs were affected with intestinal illness (diarrhoea) , while in lambs vaccinated with Ultra-corn, 2 respiratory cases and 2 intestinal disease cases were observed. In comparison to the control lambs, 4 cases showed intestinal and 3 cases were respiratory affected. The conventional treatment with antibiotics cured the diseased cases promptly.

DISCUSSION

The differential leukocytic count (Table, 1) showed a marked increase in the lymphocytes over the normal limits from the first weak up to the 4th week post-vaccination with BCG and Ultra-corn (Subgroup II B). The ingestedparticles of the immunostimulants may have stimulated the lamb lymphocytes as a foreign protein and produced those high counts which appeared during this study. It was previously postulated that when certain antigens of intracellular infections are introduced into a host, they activate the macrophages of the reticulothelial system to engulf the antigens, break them up and excrete the products (Unanue, 1972; Sell, 1978 and Rosenthal,

1980). Some determinants of the later are taken up by receptors of small lymphocytes (T cells). These are activated to large blast forms which in turn propagate into smaller, especially sensitized lymphocytes. These so called T cells represent 80% of the circulating lymphocytes and are involved in cellular immunity or delayed hypersensitivity (Williams and Waksman, 1969; Mackaness, 1971; Raffel, 1971, and Katz and Benacerraf, 1972).

Table (2) demonstrates that BCG vaccination gave clear positive skin reaction 6 months post-vaccination, if we consider an increase of 5 mm skin thickness as p 1984), while Ultra-corn vaccination gave negative reaction. Results indicated that the BCG vaccinated lambs had an efficiently stimulated cell-mediated or delayed type hypersensitivity (DTH). This relative higher cellular response and prolonged effect resulted from BCG vaccination than that shown due to Ultra-corn vaccination may be due to the living bacteria which propagate inside the animal body (Saleh et al., (1993). Rosenthal (1980) attributed that sensitized lymphocytes that will transfer immunity and hyperesentsitivty can be attained most effectively by viable cells.

Concerning humoral immune response, the significant elevation of gamma-globulin, total immunoglobulins and immunoglobulin-G (Tables, 3 and 4) in BCG and Ultra-corn (subgroup IIB) vaccinated lambs compared with nonvaccinated ones, 3 and 4 weeks post-vaccination, could be attributed to the increase in their lymphocyte counts. Halliwell and Gorman (1989) stated that immunoglobulin content of serum is produced by the B-lymphocytes as well as by the plasma cell which are derived from B-cells. The obtained results are in agreement with those reported by Saleh et al., (1993) and Shalaby et al., (1992) who found significant stimulation to both cellular and humeral responses in calves treated with BCG and Ultra-corn, respectively.

In conclusion, higher and prolonged cellular response were obtained in BCG vaccinated lambs than Ultra-corn vaccinated ones. This was quite clear from the marked increase in the lymphocyte and monocyte counts and positive skin test as well as improved health status of the vaccinated lambs.

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Table (1) : Different leucocytic count in vaccinated and non-vaccinated lambs.

Time of sampling	7 days post. vacc.						14 days post. vacc.					
LEUCOCYTE	N	L	N/L	M	E	B	N	L	N/L	M	E	B
Group I	×××× 31.57±2.47	×××× 67.43±2.22	×××× 0.47±0.06	0.86±0.15	0.14±0.13	0	×××× 36.10±2.14	×××× 62.50±2.38	×××× 0.58±0.05	1.00±0.42	0.40±0.15	0
Group II Subgr. A	× 41.00±3.86	× 58.50±3.84	× 0.70±0.13	0.33±0.19	0.17±0.15	0	39.00±4.74	58.40±4.57	0.67±0.15	1.00±0.56	1.20±0.52	0.40±0.36
Subgr.B	× 39.33±4.37	× 59.50±4.22	× 0.66±0.13	0.68±0.10	0.50±0.20		× 37.20±3.41	×× 61.00±3.27	× 0.61±0.09	1.20±0.52	0.60±0.22	0
Group III	52.67±3.65	46.16±4.28	1.14±0.15	0.67±0.45	0.50±0.31		48.50±3.08	49.50±2.50	0.98±0.11	1.33±0.51	0.67±0.30	0

Time of sampling	21 days post. vacc.						28 days post. vacc.					
LEUCOCYTE	N	L	N/L	M	E	B	N	L	N/L	M	E	B
Group I	×× 37.11±2.40	× 59.78±2.35	× 0.62±0.06	×× 2.22±0.41	0.89±0.29	0	×× 39.40±2.72	× 57.20±2.92	× 0.69±0.09	××× 2.80±0.53	0.60±0.32	0
Group II Subgr. A	42.80±5.02	55.00±4.00	0.78±0.14	0.60±0.36	1.20±0.44	0.40±0.36	43.20±2.92	54.80±3.03	0.79±0.10	1.40±0.46	0.60±0.22	0
Subgr.B	× 39.20±1.63	× 58.40±1.28	× 0.67±0.04	1.60±0.22	0.80±0.33	0	× 40.40±2.39	× 57.00±2.80	× 0.71±0.09	1.60±0.22	1.00±0.28	0
Group III	47.43±2.98	51.29±3.17	0.87±0.09	0.86±0.37	0.43±0.19	0	48.00±2.05	50.14±1.56	0.96±0.07	1.00±0.35	0.86±0.26	0

N = Neutrophil
L = Lymphocyte
N/L = Neutrophil/Lymphocyte ratio
M = Monocyte
E = Eosinophil
B = Basophil.

× = Significant at :
× = P < 0.05 ×× = P< 0.02
××× = P < 0.01 ×××× = P < 0.001

Table 2 : Tuberculin reactivity in lambs, 180 days post vaccination with BCG or Ultra-corn.

Studied groups	Skin thickness (mm) before and 72 hours after i.d. injection of 0.1 ml tuberculin		
	Before	After	difference
Group I	2.05±0.08	8.23±1.01	xx 6.18±0.98
Group II Subgr. A.	1.90±0.16	3.40±0.50	1.50±0.34
Subgr. B	2.08±0.08	4.70±0.22	x 2.62±0.26
Group III	1.79±0.11	2.98±0.41	1.19±0.42

x= significant at:
x = P < 0.01
xx = P < 0.001

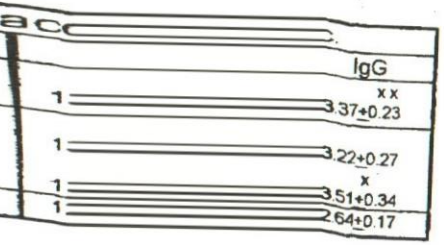
Table 3 : Difference in the percentages of gamma-globulin in vaccinated and non-vaccinated lambs.

Studied groups	14 days			21 days			28 days		
	γ %	γ1 %	γ2 %	γ globulin %	γ1 %	γ2 %	γ globulin %	γ1 %	γ2 %
Group I	29.02±0.75	20.24±0.66 ×	8.78±0.27	29.63±0.89	20.28±0.58	9.35±0.67	28.91±0.83 ××	20.33±0.56 ××	8.58±0.40 ×
Group II									
Subgr. A	26.36±0.93	18.60±0.50	7.76±0.39	27.56±0.74	19.52±0.53	8.04±0.30	27.63±0.56	20.02±0.34 ×	7.61±0.37
Subgr. B	28.28±0.84	19.64±0.61	8.64±0.37	29.75±0.81 ×	20.44±0.53	9.31±0.62	29.17±0.89 ××	20.79±0.55 ×××	8.38±0.35 ×
Group III	26.75±0.75	18.75±0.41	8.00±0.38	27.15±0.86	18.89±0.68	8.56±0.48	25.80±0.87	18.45±0.53	7.35±0.33

× = Significant at :
x = P< 0.05
×× = P< 0.02
××× = P < 0.01

Table 4 : Serum total protein (gm/dl), total immunoglobulins (gm/dl) and Immunoglobulin-G (mg/ml) in vaccinated and non-vaccinated lambs.

Time of sampling	7 days post-vacc.			14 days post-vacc.			21 days post-vacc.			28 days post-v	
Parameter	T.P.	Igs	IgG	T.P.	Igs	IgG	T.P.	Igs	IgG	T.P.	Igs
Group I	6.02±0.08	2.11±0.06	12.91±0.19	5.94±0.12	2.15±0.08	XXXX 13.19±0.17	6.13±0.08	2.26±0.06 xx	13.38±0.13	6.20±0.13 x	2.20±0.06
Group II											
Subgr. A	5.84±0.21	2.07±0.14	12.67±0.10	6.07±0.11	2.16±0.10	13.11±0.34	6.07±0.20	2.15±0.11	13.26±0.26	6.10±0.24	2.16±0.08
Subgr. B	5.99±0.11	2.15±0.11	12.76±0.22	6.17±0.11	2.23±0.14	13.04±0.44	6.40±0.15	2.30±0.12 x	3.55±0.33 x	6.32±0.12 xxx	2.21±0.06 x
Group III	5.88±0.12	1.98±0.08	12.61±0.20	6.01±0.07	2.04±0.09	12.35±0.13	6.10±0.10	1.96±0.09	12.57±0.26	5.77±0.13	1.95±0.13



T.P. = Serum total protein
Igs = Total Immunoglobulins
IgG = Immunoglobulin-G

× = Significant at :
× = P< 0.05
×× = P< 0.02
××× = P < 0.01