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EFFECTS OF *CORYNEBACTERIUM CUTIS* EXTRACT THERAPY ON THE IMMUNE RESPONSE OF BRUCELLA - VACCINATED BUFFALO CALVES

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ABSTRACT

The effects of *Corynebacterium cutis* extract on lymphocyte transformation, phagocytic activity and antibody level were studied in 4-months-old buffalo calves vaccinated against *Brucella abortus* using strain 19 vaccine. The results revealed that treatment with *C. cutis* extract, either 3 days prior to or concurrently with the vaccine evoked a significant elevation of lymphocyte stimulation indices in the presence of phytohaemaglutinine, concanavaline-A and *Brucella abortus* soluble antigen as well as significant increase in the phagocytic activity of mononuclear cells against *Candida albicans*. Antibody levels were estimated by rivanol and serum tube agglutination test. Administration of *C. cutis* extract before or simultaneously with the vaccine provoked a marked elevation of antibody titre. Conversely, *C. cutis* injection one week postvaccination, did not elicit any important alterations in either cell mediated or humoral immune responses.

INTRODUCTION

Brucellosis is a highly contagious disease affecting a wide variety of animals. It is a major source of serious economical losses due to abortion and decreased milk production. Strain 19, Brucella abortus vaccination of young calves provides a certain degree of protection against the disease, which is, however, incomplete and of short duration, especially with reduced dose schedules[1].

The use of immunostimulant agents together with Brucella vaccine might be more effective than the vaccine alone for stimulation of the immune system, augmentation of the immune response and raising animal resistance to infection [2-5].

The present study was designed to evaluate the effect of *Corynebacterium cutis* therapy in Brucella vaccinated buffalo calves.

MATERIAL AND METHODS

Immunostimulant:

A complete -lysate of *Corynebacterium cutis* bacterial extract (Ultracorn, Virbac laboratories-France) in a concentration of 20 mg/ml was the immunostimulant used.

Animals used:

Twenty four female bufflao calves 4-months old, were used. They were divided into four equal groups. *C. cutis* extract was administered im (2ml/ 100 kg body weight) either 3 days pre-vaccination (Group I), concurrently with the vaccine (Group II), or one week post-vaccination (Group III). The Fourth group received the vaccine (Brucella abortus strain 19, Coopers Animal Health Inc., Kansas, USA) alone and served as a control.

Blood samples were taken one week pre-vaccination as well as 1, 2, 3, 4, 6, 8, 12 and 16 weeks post-vaccination.

The cellular immune response of treated and control calves was assessed by the lymphocyte transformation. Blast transformation of peripheral blood lymphocytes was measured according to previously reported methods[6,7]. The lymphocytes were separated using ficoll, washed three times then suspended in RPMI-1640 media and 10% fetal calf serum. The lymphocytes were cultured with Phytohaemagglutinine (PHA, 19mg/ml) or Concanavaline A (Con. A, 15 mg/ml) or Brucella soluble antigen* (BSA, 5mg/ml). The plates were incubated for 48-72 hr. in 10% CO2 incubator Thereafter, the residual glucose consumed in PRMI-1640 media used in this technique was determined according to the equation given by Charles et al.,[8]. In addition, phagocytosis assay was performed according to Richardson and Smith [9]. The percentage of mononuclear phagocytic cells and phagocytic indices were determined by the ability of these cells to engulf *Candida albicans*.

For studying the humoral immune respnse, the titer of antibodies was measured in serum using serum tube agglutination test[10]. Simultaneously, rivanol test was carried out according to the procedures of the National Veterinary Services Laboratories, Ames, Iowa, USA[11].

The obtained results were subjected to Student's (t) test to reveal the significance of differences between treatment and control groups [12].

RESULTS

The obtained results presented in Table 1, show that treatment with C. cuties extract 3 days pre-vaccination

(group I) caused a significant increase in lymphocyte stimulation indices that were recorded after the first week of vaccination and lasted throughout the whole period of the experiment.

Similarly, concomitant administration of *C. cutis* extract and Br. abortus strain 19 vaccine (group II) evoked a long lasting significant increase in lymphocyte blastogenesis indices by the first week of vaccination. The previous increase lingered till the 12th week. On the 16th week of vaccination, normal control values were observed as compared with the non treated vaccinated group (group IV).

However, calves treated with *C. cutis* extract one week post-vaccination (group II), exhibited no significant changes in lymphocyte stimulation indices in comparison with the non-treated vaccinated group.

Data depicted on Table 2 indicate that animals given *C. cutis* extract 3-days pre-vaccination or on the same day of vaccination exhibited a significant augmentation of phagocytic cell percent and phagocytic indices after the first week post-vaccination that lasted throughout the rest of experiment. Meanwhile, *C. cutis* extract administration one week post-vaccination induced no significant changes as compared with the non-treated vaccinated group.

As shown in Table 3, the *C. cutis* extract-treated group 3 days pre-vaccination, displayed a marked elevation of antibody titer after the second week of vaccination that lingered through the fourth week of vaccination. Concomitant administration of *C. cutis* extract and *Br. abortus* strain 19 evoked a significant increase of antibody level after two weeks that extended to the fourth week of vaccination.

On the sixth week, the antibody titer returned to the normal control levels as compared with the nontreated vaccinated group. Meanwhile, treatment with *C. cutis* extract on the seventh day post-vaccination did not generate any significant increase of antibody titers in comparison with the non-treated vaccinated group.

DISCUSSION

The use of immunostimulants such as levamisole along with vaccination has been believed to lead to an increased protection against brucellosis [13].

Administration of *C. cutis* extract (ultracorn) prior to vaccination or simultaneously with vaccine evoked a significant increase of lymphocyte stimulation indices. Paradoxically, post vaccination treatment with *C. cutis* extract provoked no significant changes compared with the non-treated vaccinated group.

* Br. abortus soluble antigen (BSA) was kindly obtained from the Department of Large Animal clinical Sciences, college of Veterinary medicine, University of Magnesota, St Paul, USA

It is tempting to suggest that *C. cutis* extract stimulated lymphocyte index by virtue of its stimulant effect on the synthesis of lymphocyte DNA which could be due to the presence, in *C. cutis* extract preparation, of lipopolysaccharides or glycopeptides that stimulate blastogenesis[14]. On the other hand, as previously found with *C. parvum*, *C. cutis* extract might have stimulated the lymphoreticular system in general, and enhanced T lymphocyte activity [15, 16]. These results substantiate those obtained before in cattle[17] and in chickens [18, 19].

In the present study, the percentage of mononuclear phagocytic cells and phagocytic indices were detemined by their ability to engulf the *Candida albicans*. Administration of *C. cutis* extract on the day of vaccination or 3 days pre-vaccination resulted in a significnat increase of phagocytic activity in comparison with the non-treated vaccinated group. In this context, it is suggestive that neutrophils may play a role in macrophage activation by releasing metabolic product(s) in animals treated with Corynebacterium suspension[19].

A relevant reason could b through stimulation of reticuloendothelial system represented by T cells, that release their metabolic products thereby activating macrophages [21,22].

The results obtained on the effect of *C. cutis* extract on phagocytic activity are in accordance with those previously reported by some authors [23, 24] who indicated that the corynebacteria provoked an increase of phagocytic activity of monocytes of healthy bovine calves. Moreover, [25-27] recorded significant elevation of phagocytic cells after treatment of buffalo calves and Friesian cows with *C. cutis* extract.

The results of the present work indicate that *C. cutis* extract treatment was beneficial for significantly potentiating higher antibody titre especially in claves treated 3 days before vaccination or on the day of vaccination.

The improved response of calves to treatment with *C.cutis* extract along with vaccination could be explained by the fact that killed Corynebacterium suspension significantly stimulates both T and B lymphocyte activity with consequent increase in antibody formation[18,29].

These results are in agreement with those previously obtained[3]. Who reported that immunization against H. rhusiopathiae increased the vaccinal potency 1.35-2.15 times when given with such vaccine. Moreover, some authors reported an increase of antibody titer against Newcastle disease virus vaccine when ultracorn was

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Table (I): Effect of ultracorn stimulation indices (in the presence of PHA, Con-A and BSA) in vaccinated bufflao calves. (Mean S.E).

Treatment	Group I			Group II			Group III			Group IV		
	PHA	Con-A	BSA	PHA	Con-A	BSA	PHA	Con-A	BSA	PHA	Con-A	BSA
Prevaccination	1.58 ± 0.18	1.37 ± 0.13	1.28 ± 0.16	1.73 ± 0.23	1.54 ± 0.17	1.52 ± 0.21	1.73 ± 0.18	1.82 ± 0.32	1.52 ± 0.15	1.48 ± 0.16	1.28 ± 0.25	1.29 ± 0.22
1 w p.v.	2.93 ±0.32*	2. 83 ± 0.26 *	2.46 ± 0.13 *	2.88 ± 0.26 *	2.93 ± 0.19 *	2.85 ± 0.23*	1.98 ± 0. 32	1.52 ± 0.16	1.61 ± 0. 16	1.24 ± 0.39	1.85 ± 0.23	1.63 ±0.21
2 w p.v.	4.52 ± 0. 31 *	3. 82 ± 0.31*	3.85 ± 0. 16 *	3.95 ± 0.16 *	3.52 ± 0.26 *	3.85 ± 0.16 *	3.27 ± 0. 33	2.62 ± 0. 27	3.21 ±0. 32	3.33 ± 0. 27	2.32 ± 0.27	3.13 ± 0.26
3 w p.v.	5.36 ± ± 0. 51*	3.93 ± 0.03 *	5.02 ± 0.52 *	3.89 ± 0.13 *	4.17 ± 0.31*	4.23 ± 0.02 *	3.52 ± 0. 53	3.86 ± 0.39	3.91 ± 0.41	3.42 ± 0. 25	3.36 ± 0. 39	3.08 ± 0.47
4 w p.v.	5.63 ± 0. 42 *	4.52 ± 0.24 *	4.72 ± 0.27 *	4.95 ± 0.15 *	4.25 ± 0.15 *	4.56 ±0. 11 *	3.95 ± 0. 41	3.52 ± 0. 35	3.82 ± 0.39	4.37 ± 0.45	3.17 ± 0.32	3.71 ± 0. 36
6 w p.v.	4.52 ± 0. 24 *	4.62 ± 0.26 *	4.18 ± 0.42*	5.07 ± 0.35 *	4.53 ± 0.22 *	3.87 ± 0.13 *	3.76 ± 0. 35	3.66 ± 0. 37	3.11 ± 0. 31	3.62 ± 0.32	3.25 ± 0.32	3.05 ± 0.36
8 w p.v.	4.54 ± 0. 42 *	5.02 ± 0.41 *	5.11 ± 0.35 *	5.13 ± 0.31 *	4.62 ± 0.16 *	4.83 ± 0.17 *	3.41 ± 0. 46	3.75 ± 0. 38	4.07 ± 0. 40	3.07 ± 0.36	3.85 ± 0.35	3.86 ± 0.31
12 w p.v.	5.23 ± 0. 56 *	5.15 ± 0.51 *	4.83 ± 0.0. 5 *	5.06 ± 0.46*	4.15 ± 0.18*	4.95 ± 0.21 *	3.51 ± 0. 36	3.63 ±0.36	4.23 ± 0.42	3.09 ± 0.33	3.51 ± 0. 33	4.08 ± 0.31
16 w p.v.	5.67 ± 0.63 *	4.28 ± 0.47 *	3.93 ± 0.32 *	4.01 ±0.15 *	3.87 ± 0.15*	3.95 ± 0.21*	3.36 ± 0. 34	3.28 0. 33	3.11 ± 0. 31	3.25 ± 0. 27	3.09 ± 0.31	3.02 ± 0.15

w p.v. Week post-vaccination

* Significant from corresponding control value at $P < 0.05$

Table (2): Effect of ultracorn on the phagocytic % (PHAG%) and phagocytosis indices (PHAG. INDEX) in vaccinated buffalo calves. (MEan S.E).

Treatment	Group I		Group II		Group III		Group IV	
	PHAG. %	PHAG. INDEX	PHAG. %	PHAG. INDEX	PHAG.%	PHAG. INDEX	PHAG. %	PHAG INDEX
Prevaccination	46.2 ± 4.5	1.36 ± 0.32	45.2 ± 4.5	1.30 ± 0.37	42.6 ± 4.2	1.36 ± 0. 25	41.5 ± 0. 6	1.31± 0. 18
1 w p.v.	63.5 ± 4.32*	3.26 ± 0.33 *	59.5 ± 3.36 *	1.83 ± 0. 15 *	45.2 ± 4. 45	1.52 ± 0. 26	43.7 ± 4. 72	1.26 ± 0. 13
2 w p.v.	67.2 ± 3.56 *	2.65 ± 0.21*	67.9 ± 2.52 *	2.67 ± 2.26*	57.2 ± 3.33	1.62± 0. 25	55.0 ± 5. 86	1.28 ± 0. 15
3 w p.v.	65.3 ± 3.33 *	3.33 ± 0.26 *	73.5 ± 5.85 *	2.56 ± 0.23 *	58.3 ± 5. 86	1.46 ± 0. 15	53.3 ± 5. 22	1.29 ± 0. 11
4 w p.v.	68.8 ± 1.68 *	2.82 ± 0.29 *	70.2 ± 2.5 *	2.42 ± 0.15 *	56.2 ± 5. 26	1.37 ± 0. 23	58.2 ± 5.5	1.17 ± 0. 12
6 w p.v.	69.7 ± 2.35 *	2.67 ± 0.27 *	68.7 ± 1.23 *	2.36 ± 0.23 *	59.65. ± 68	1.53 ± 0. 18	58.63 ± 4. 18	1.27 ± 0. 13
8 w p.v.	63.3 ± 1.56 *	2.56 ± 0.24 *	67.3 ± 4.26 *	2.44 ± 0.15 *	53.5 ± 5. 51	1.25 ± 0. 15	52.4 ± 5. 6	1.35 ± 0. 10
12 w p.v.	71.5 ± 3.46 *	2.86 ± 0.21 *	73.3 ± 5.83 *	2.50 ± 0.29 *	51.4 ± 4. 56	1.60 ± 0. 28	48.6 ± 5. 01	1.26 ± 0. 09
16 w p.v.	65.6 ± 3.66 *	1.97 ± 0.18 *	62.3 ± 3.33 *	2.36 ± 0.21 *	52.5 ± 5. 38	1.39 ± 0. 21	51.2 ± 4. 31	1.18 ± 0. 01

w p.v. : week post-vaccination

* : Significant from corresponding control value at $P < 0.05$.

Table (3): Effect of ultracorn on the antibody titre using Rivanol (RIV.) and standard agglutination test (SAT) in vaccinated buffalo calves (Meam S.E).

Treatment	Group I		Group II		Group III		Group IV	
	SAT	*RIV*	*SAT*	*RIV*	*SAT*	*RIV*	*SAT*	*RIV*
Prevaccination	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
1 w p.v.	86.3 ± 0.0	25.0 ±0.0	81.0 ± 12.5	25.0 ±0.0	70.2 ±10.8	25.0 ±0.0	66.0 ±10. 3	25.0 ± 0. 0
2 w p.v.	513.82 ± 54.8 *	200.1 ± 30. 8*	457.33 ± 56.7*	200.1 ± 20.7*	301.5 ± 21.7	100 ± 10.8	299.3 ± 35. 2	90.0 ± 10. 5
3 w p.v.	1083.7 ± 86. 5*	405.8 ± 81. 5 *	1113.58 ±86.7*	388.5 ± 40.7*	875.8 ± 85.8	160. ± 15.3	897 ± 91.6	160.0 ± 15. 3
4 w p.v.	802.56 ± 60.43*	705.8 ± 71.48*	810.59 ± 90.7 *	750.72± 100. 3*	436.4 ± 51.7	400 ± 46.0	427 ± 52.2	400.0 ± 46. 0
6 w p.v.	405.28 ± 48.5	250.73 ± 40.71	417.83 ± 50.7	380.0 ± 50. 3	375.2 ± 37.5	200 ± 20.0	382.6 ± 35.1	200.0 ± 20. 0
8 w p.v.	310.33 ± 45.7	180.58 ± 21.3	315.72 ± 44.5	200 ± 30.6	280.7 ± 36.7	100 ± 20.3	245 ± 25.7	150.0 ± 15.0
12 w p.v.	103.26 ± 18.5	39.38 ± 5.8	117.85 ± 20. 5	50. 6 ± 10.3	85.63 ± 8.8	25 ± 0.0	90.81 ± 9.52	30.0 ± 5.6
16 w p.v.	70.35 ± 10.5	25.0 ± 0.0	65.51 ± 7.8	25.0 ± 0.0	52.3 ± 5.7	25 ± 0.0	59.2 ± 6.17	25.0 ± 0. 0

w p.v. : Week post-vaccination

* : Significant from corresponding control value at $P < 0.05$.

given with vaccination [18,31].

If one were to assume a priming effect of C. cutis extract on the body immune responsiveness to subsequent expsure to *Br. abortus* strain 19 vaccine, then it could be surmised that this putative effect is only at work when *C. cutis* extract is given prior to or simultaneously with the vaccine. In favour of the previous conjuncture, is the fact that *C. cutis* extract given post vaccination is no longer effective in potentiating its immunomodulating activity.

Future studies are needed to address the issue whether *C. cutis* extract administration is effective or not in providing a reliable protection against Brucella infection.

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تأثير العلاج بخلاصة الكوارينباكتريوم كيوتس على الاستجابة المناعية فى عجول الجاموس المحصنة ضد مرض البروسيلا

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تمت دراسة تأثير العلاج بخلاصة الكوارينباكتريوم كيوتس على تحول الخلايا الليمفاوية، النشاط الإلتهامى والأجسام المضادة فى عجول الجاموس المحصنة ضد مرض البروسيلا بلقاح العترة رقم ١٩.

وأظهرت النتائج أن العلاج بخلاصة الكوارينباكتريوم كيوتس لمدة ٣ أيام قبل أو مع اللقاح أحدث زيادة معنوية فى معامل تنشيط الخلايا الليمفاوية فى وجود الفيتوهيم أجليوتينين والوكنكانا-فالين-أ وأنتيجين البروسيلا المجهض الذائب بالإضافة إلى زيادة معنوية فى النشاط الإلتهامى للخلايا وحيدة النواه ضد فطر الكانديدا ألبيكان.

وقد تم تقدير مستوى الأجسام المضادة المناعية بواسطة الريفانول واختبار تلازن المصل فى الأنابيب. ولقد زاد مستوى هذه الأجسام المناعية عندما أعطيت خلاصة الكوراين بكتريوم كيوتس قبل وأثناء التحصين، بينما لم تحدث أية تغيرات معنوية فى الاستجابة المناعية الخلوية أو العضوية عندما تم حقن خلاصة الكوارين بكتريوم كيوتس قبل التحصين بأسبوع.