EXPERIMENTAL MODULATION OF VACCINAL IMMUNITY OF BOVINE BRUCELLOSIS

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ABSTRACT

Brucellosis has been reported as one of the important causes of considerable economic losses due to reduction in production. Vaccination is generally not practiced except in large infected organized and state owned farms. Segregation and quarantine of infected animals is not economical. Alternative is the immuno potentiation along with the vaccination for control programme. Hence, in this stud, humoral antibody response was evaluated using standard tube agglutination test and cell mediated immunity using contact sensitivity in different groups of cattle and buffalo calves receiving levamisole injection and chelated zinc along with vaccination against brucellosis. The results revealed that levamisole treatment significantly augmented humoral response. Histomorphological studies indicated higher intensity of changes like hyperkeratosis, leukocytic influx and fibroplasias pointing out delayed hypersensitivity reaction. Globulin fraction of total protein showed increasing trend in levamisole treatment group. The results indicated that immuno potentiation should be adopted to achieve better vaccinal response.

KEY WORDS: Brucellosis, bovine, dinitrochlorobenzene, immuno potentiation, skin fold thickness.

INTRODUCTION

Economic losses through reproductive disorders associated with brucellosis are quite high. Brucellosis has been reported as one of the important causes of considerable economic losses due to reduction in production (Michel, 2003). It is estimated that an abortion would cost the farmer Rs. 5000/- which can be avoided by initializing control measures. Segregation and quarantine of infected animal is uneconomical. Hence, an alternative approach is to increase the herd immunity through vaccination. Vaccination in India is generally not practiced except in the large, infected organized, state owned farms (Renukaradhya *et al.*, 2002). Immuno potentiation along with vaccination can help to the control programme. Hence, this paper places on record evaluation of the effect of immuno potentiation in brucella vaccinated calves. Present trial was conducted with a view to ascertain better ways of vaccination to afford better protection.

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MATERIALS AND METHODS

Thirty six female calves of Kankrej cattle and Mehsana buffalo aged 4-8 months, weighing 100-120 Kg. maintained at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujatrat, were selected and divided in to three groups. Each group comprised of six cattle calves and six buffalo calves. Group I was treated as control, calves in this group received vaccine *Bruvac** @ 2 ml sub cutaneously (*Bruvac** containing *Brucella abortus s- 19* vaccine manufactured by Indian Immuniologicals Ltd.; Hyderabad). Group II calves were injected with levamisole Hcl intramuscularly thrice at 48 Hr. interval from the day of vaccination. Group III calves were given chelated zinc @ 300 mg/day/head for 40 days before and 60 days after vaccination. Blood was collected on 0, 15, 30 and 60 days post vaccination for separation of serum.

Humoral antibody response was measured on 0, 15, 30 and 60 days post vaccination by Standard Tube Agglutination Test (STAT). Cell mediated immune response was assessed by contact sensitivity to challenged i.e. II topical application of 2, 3 dinitroclorobenzene (DNCB) 15 days after primary sensitization on contra lateral side. Primary sensitization with 0.5 ml of 2 % DNCB in acetone was done on 15 day post vaccination. The skin thickness was recorded at 24, 48 and 72 hour post challenge application of DNCB. Skin biopsy samples of 4 mm diameter were collected 24 Hr and 48 Hr. post challenge application as per the method adopted by Reddy *et al.*, 1981. Skin biopsy samples were processed and stained by Ehrlich's haematoxylene and eosin technique (Luna, 1968). Histomorphological evaluation was conducted based on histological structure of buffalo skin (Bagi, 1974). Total protein and albumin were estimated by using kit manufactured by Span diagnostics. Serum zinc was estimated by Atomic Absorption Spectrophotometry. Data were transformed to perform non parametric analysis by allocating numerical weightage to the serum dilution indices as below and the data were analyzed using "t "test.

Sr. No.	Dilution Factor	Score on a 4.0 scale
1	1:10	0.25
2	1:20	0.50
3	1:40	1.00
4	1:80	2.00
5	1:160	4.00

(Positive reaction with zero dilution was not considered).

RESULTS AND DISCUSSION

By STAT, humoral antibody titres elevated 15 days post vaccination in all the three groups and thereafter, started declining 30 days after vaccination. The dilution factor when transformed with numerical scores on time scale and presented in percentage, the antibody responses were explained better (Table 4).

In all the groups, antibody titre showed tapering trend with the advancement of time. However, observations indicated that the levamisole treatment (Group- II) and zinc supplementation (Group- III) calves had enhanced humoral antibody

response (Table 2 & Table 3). Majority of the calves of group- II followed by Group- I maintained antibody level in serum at 60 days post vaccination. Levamisole treatment significantly augmented humoral response. Humoral antibody response in zinc supplementation calves was higher than control calves. This indicated augmentation in humoral antibody in Group- III. However, immunomodulating effect was lesser compared to levamisole group. Mineral mixture containing trace elements like zinc are relatively cheaper, available and easy to administer so; supplementation of mineral mixture in remote and backward areas can promote immune response.

Cell mediated immunity evaluated on the basis of increase in skin fold thickness (SFT) (Table 4) revealed that the mean increment attained peak at 24 Hr. post challenge .In group- I of cattle and buffalo calves, increase in SFT was non significant while in calves of Group- II and Group- III, SFT at 24 Hrs. was 6.21 to 8.24 mm and 6.29 to 6.95 mm; respectively in cattle. Corresponding figures for buffalo calves were 9.01 to 10.72 and 10.08 to 10.92.

Challenge application of DNCB on 15 days after primary application revealed marked erythema, swelling and oedema at 24 Hrs. and these changes subsided at 48 Hrs. Histological examination of the skin biopsy from challenged site after 24 Hrs. revealed dermal oedema and leukocytic infiltration (Plate I to III),. At 48 hours, histological changes were dermal oedema, mild leukocytic infiltration, fibrous and connective tissue proliferation, sclerosis of blood vessel and hyper keratosis. These changes were more pronounced to Group- I (Plate IV to VI). Skin fold thickness increased significantly ($P< 0.05$) 24 hours after challenge application of DNCB in levamisole group. Histomorphological changes 48 Hrs. post challenge characterized by fibrous hyperkeratosis, increased leukocytic influx and fibroplasias were indicative of the ongoing allergic dermatitis projecting stimulated cell mediated immune response. Histomorphological examination revealing higher intensity in levamisole treated group calves pointing out delayed hypersensitivity reaction.

Mean values of Total protein, Albumin, Globulin, and Zinc showed non significant changes in all the three groups (Table 5). Globulin fraction of Total protein show increasing trend from the day if vaccination to 30 days post vaccination with relative decreased albumin.

Present findings with reference to the pattern of antibody response up to 30 days post vaccination in cattle and buffalo calves were similar to the findings of Poester and Reckziegel, 1998 and Singh *et al.*, 2002. Antibody titre tapered with the advancement in time. Results indicated maximum humoral antibody response in calves receiving levamisole treatment followed by calves receiving chelated zinc and control calves. Thus, augmentation in humoral antibody response was significantly higher in levamisole treatment group. Similar findings were reported in cattle (Chukwu, 1987) and buffaloes (Qureshi *et al.*, 2000). Findings of higher humoral response in zinc supplemented calves corroborate findings of Yadav *et al.*, 1998. Immuno potentiating effect of chelated mineral mixture has been documented (Rao *et al.*, 1986) in growing Kankrej calves vaccinated with Bruvac. Immune system like thymus stimulatory activity of zinc has

well established (Tizzard, 1998, Girodon *et al.*, 1999 and Singh *et al.*, 2012). Increment in SFT was higher (2.03 mm in cattle & 1.71 mm in buffalo calves) in levamisole treatment group than the calves of group III (0.66 mm in cattle & 0.84 mm in buffalo calves) receiving chelated zinc suggesting better cell mediated immune response in levamisole treated group of calves. Skin fold thickness increased significantly ($P < 0.05$) 24 Hrs. after challenge application of DNCB in levamisole treatment group which might be due to delayed type of cellular immunoreactivity. Similar findings were reported in mice (Taylor *et al.,* 1993). Though biochemical parameters showed non significant changes in all the three groups of cattle and buffalo calves, Globulin fraction of total proteins showed increasing trend from the day of vaccination to 30 days post vaccination with relative decreased albumin in cattle calves. Increased globulin levels were attributed to synthesis of Immunoglobin following vaccination (Tizzard, 1998).

CONCLUSION

From the results, it can be concluded that levamisole treatment significantly augmented humoral response. Histomorphological studies indicated higher intensity of changes like hyperkeratosis, leukocytic influx and fibroplasias pointing out delayed hypersensitivity reaction. Globulin fraction of total protein showed increasing trend in levamisole treatment group. The results indicated that immuno potentiation should be adopted to achieve better vaccinal response.

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Table 1: Profile of weighted score card of antibody response to *Brucella abortus* vaccinated experimental Kankrej and Mehsana calves.

Group	Weighted Score in Percentage		
	15 day	30 day	60 day
Kankrej Calves			
Group-I	33.33	17.70	3.13
Group-II	83.33	62.50	33.33
Group-III	52.08	39.58	19.79
Mehsana Calves			
Group-I	43.75	18.75	3.13
Group-II	68.75	54.17	37.50
Group-III	50.00	37.50	14.58

Table 2: Humoral immune response observed in immunomodulated experimental Kankrej calves following immunization with *Brucella abortus* S- 19 vaccine.

Antibody titre Group	0	1:10	1:20	1:40	1:80	1:160
On 15 day post vaccination						
Group-I	0	2	1	1	1	1
Group-II	0	-	-	-	2	4
Group-III	0	-	1	2	1	2
On 30 day post vaccination						
Group-I	1	1	2	1	1	-
Group-II	-	-	-	1	3	2
Group-III	-	-	1	3	1	1
On 60 day post vaccination						
Group-I	4	1	1	-	-	-
Group-II	-	-	2	1	3	-
Group-III	1	1	1	2	1	-

Note: Calves show no antibody titre on the day of vaccination

Table 3: Humoral immune response observed in immunomodulated experimental Mehsana buffalo calves following immunization with *Brucella abortus* S- 19 vaccine.

Antibody titre Group	0	1:10	1:20	1:40	1:80	1:160
On 15 day post vaccination						
Group-I	-	-	1	2	2	1
Group-II	-	-	1	-	2	3
Group-III	-	-	-	2	3	1
On 30 day post vaccination						
Group-I	1	2	2	1	1	-
Group-II	-	-	-	3	2	1
Group-III	-	-	2	2	1	1
On 60 day post vaccination						
Group-I	4	1	1	-	-	-
Group-II	-	-	1	1	4	-
Group-III	-	2	2	2	-	-

Note: Calves show no antibody titre on the day of vaccination

Table 4: Profile of cutaneous contact sensitivity to challenge application of 2, 4 dinitrochlorobenzene.

Groups	Skin Fold Thickness (mm)		
	0 hrs (n=6)	24 hrs (n=6)	48hrs (n=3)
Kankrej Calves			
Group-I	7.73 ± 0.70	8.60 ± 0.76	7.89 ± 0.66
Group-II	6.21 ± 0.66	8.24* ± 0.57	6.53 ± 0.53
Group-III	6.29 ± 0.49	6.95 ± 0.49	6.82 ± 0.44
Mehsana Calves			
Group-I	7.57 ± 0.55	8.20 ± 0.52	7.23 ± 0.40
Group-II	9.01 ± 0.56	10.72* ± 0.57	9.29 ± 0.73
Group-III	10.08 ± 0.58	10.92 ± 0.45	9.92 ± 0.58

** Significant at (P<0.05)*

Table 5: Biochemical changes observed in immunomodulated calves following immunization with *Brucella abortus* S- 19 vaccine

Parameter	Interval (days)	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)
Kankrej Calves				
Total protein mg%	0	7.01 ± 0.17	7.09 ± 0.13	7.53 ± 0.18
	15	7.07 ± 0.18	7.11 ± 0.11	7.49 ± 0.10
	30	7.36 ± 0.16	7.63 ± 0.13	7.62 ± 0.12
	60	7.17 ± 0.30	7.28 ± 0.10	7.94 ± 0.13
Albumin mg%	0	3.70 ± 0.14	3.88 ± 0.10	4.20 ± 0.10
	15	3.91 ± 0.16	3.77 ± 0.12	3.94 ± 0.05
	30	3.64 ± 0.18	3.84 ± 0.06	3.91 ± 0.16
	60	3.61 ± 0.20	4.12 ± 0.73	4.24 ± 0.05
Globulin mg%	0	3.31 ± 0.16	3.21 ± 0.10	3.33 ± 0.13
	15	3.15 ± 0.20	3.34 ± 0.14	3.55 ± 0.11
	30	3.72 ± 0.08	3.80 ± 0.11	3.72 ± 0.15
	60	3.46 ± 0.22	3.16 ± 0.08	3.70 ± 0.12
Zinc ppm	0	2.44 ± 0.73	2.30 ± 1.02	3.91 ± 0.31
	15	2.35 ± 0.70	2.16 ± 1.06	3.76 ± 1.11
	30	2.28 ± 0.99	2.39 ± 1.05	3.00 ± 1.84
	60	2.30 ± 0.67	2.60 ± 1.37	3.41 ± 1.35
Mehsana Calves				
Total protein mg%	0	6.80 ± 0.11	7.04 ± 0.10	6.95 ± 0.14
	15	6.40 ± 0.16	6.77 ± 0.11	7.30 ± 0.10
	30	6.51 ± 0.11	7.57 ± 0.11	6.91 ± 0.15
	60	7.10 ± 0.25	6.92 ± 0.20	6.92 ± 0.15
Albumin mg%	0	3.25 ± 0.11	3.88 ± 0.05	3.26 ± 0.11
	15	3.01 ± 0.17	3.66 ± 0.12	3.60 ± 0.12
	30	3.40 ± 0.16	3.78 ± 0.10	3.36 ± 0.19
	60	3.54 ± 0.13	3.93 ± 0.14	3.23 ± 0.13
Globulin mg%	0	3.55 ± 0.09	3.16 ± 0.08	3.69 ± 0.21
	15	3.39 ± 0.31	3.11 ± 0.11	3.70 ± 0.15
	30	3.11 ± 0.25	3.79 ± 0.11	3.55 ± 0.11
	60	3.56 ± 0.19	2.99 ± 0.33	3.69 ± 0.17
Zinc ppm	0	2.11 ± 0.98	2.39 ± 0.77	3.90 ± 0.31
	15	2.29 ± 0.85	2.36 ± 0.71	3.09 ± 0.33
	30	2.26 ± 0.96	2.61 ± 0.88	3.57 ± 0.45
	60	2.36 ± 0.66	2.69 ± 0.65	3.72 ± 0.96

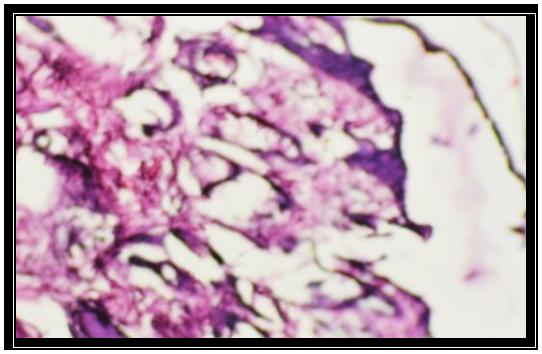

Plate I : Dermal edema and leucocytic infiltration at 24 hour.

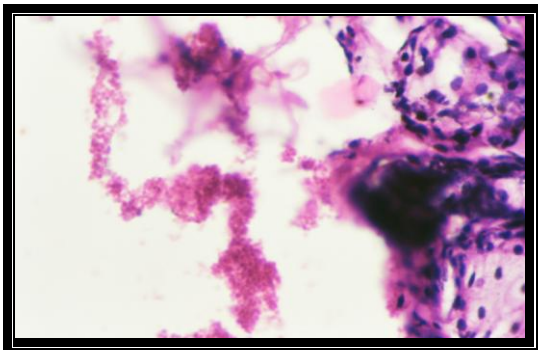

Plate II:Dermal edema and leucocytic infiltration 24 hour also note focal areas of hemorrhages

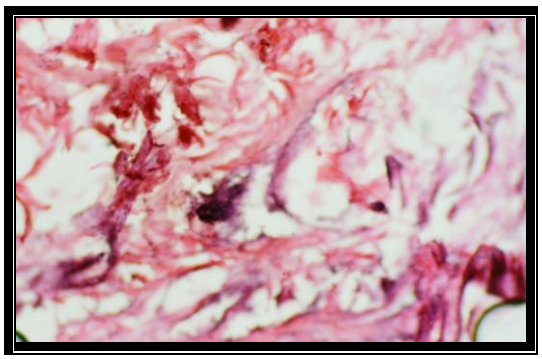

Plate III:Dermal edema and leucocytic infiltration 24 hour DNCB post challenge in zinc supplemented group.

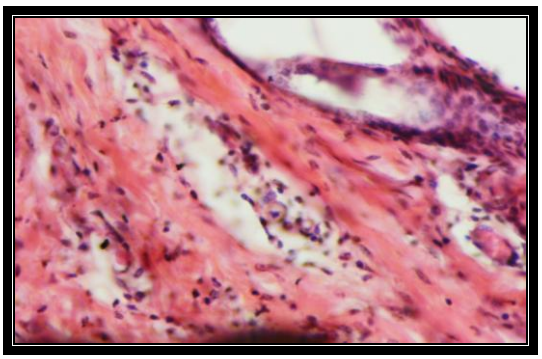

Plate IV: Dermal edema and leucocytic infiltration at 48 hour

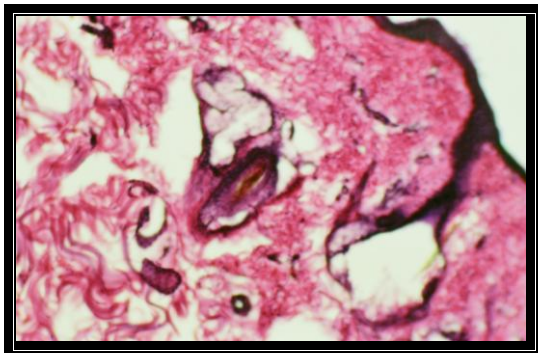

Plate V: Dermal edema and leucocytic infiltration, fibrous and connective tissue proliferation also note hyperkeratosis 48 hour post DNCB challenge in levamisole group

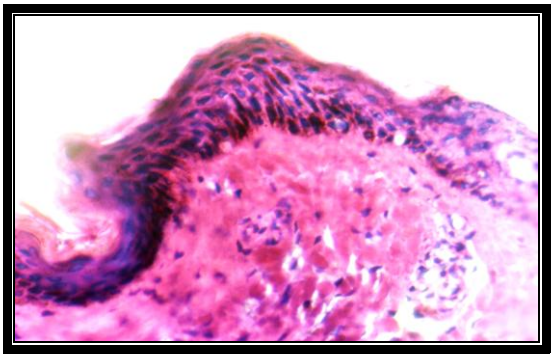

Plate VI: Sub epidermal edema and leucocytic infiltration, 48 hour post DNCB challenge in levamisole group

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