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Safety and efficacy of reduced dose *Brucella abortus* strain- 19 vaccine in non-pregnant zebu cattle (*Bos indicus*) heifers

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ABSTRACT

In India, brucellosis a re-emerging disease, is of public health and economic concern to dairy industry. *Brucella abortus* strain-19 vaccine is recommended as most effective vaccine for cattle and buffaloes, however application of full dose is restricted in adults due to persistence of antibody response that interfere with serological diagnosis. In the present study 120 seronegative female cattle (60 calves and 60 young heifers each in a group) were subjected for vaccination. In each age group, 40 animals were vaccinated subcutaneously with a reduced dose of *B. abortus* S-19 vaccine, 10 animals with standard dose and 10 animals served as controls. Animals were observed for local or systemic reactions after vaccination as well as during study period. Blood samples were collected on day 0, 21, 60, 90 post-vaccination for antibody response and cell mediated immunity (CMI). The study revealed that the reduced dose vaccine is safe with satisfactory humoral and CMI response comparable to that of the standard dose, in both the age groups of animals when administered subcutaneously. It was concluded that the *B. abortus* S-19 reduced dose vaccine is safe and effective in the young and non-pregnant cattle up to the age of 30 months.

Key words: Antibody response, *Brucella abortus* strain- 19, Brucellosis, Cell mediated immunity, Interferon-gamma assay, Reduced dose, Zebu cattle

India has a policy for the control of brucellosis in dairy cattle (Renukaradhya *et al.* 2002). As there is ban on test and slaughter programme, vaccination is considered as the main control strategy for which *Brucella abortus* strain-19 vaccine is used (Neha *et al.* 2014, Verma *et al.* 2014). It is recommended to immunize female calves at 4-8 months of age with full dose of bacteria. However, vaccination of replacement animals or calf-hood vaccination is not enough to control the disease in countries with high prevalence and uncontrolled movements of animals. In such conditions, mass vaccination programme may be effective. Application of full dose vaccine is restricted in adults due to persistence of antibody response that interfere with serological diagnosis. To overcome this drawback and limitations, reduced dose of *Brucella abortus* strain-19 vaccine was introduced. Therefore, the present study was designed to evaluate safety and efficacy of *Brucella abortus* strain-19 reduced dose vaccine and to compare the humoral and cellular response with that of *Brucella abortus* strain-19 standard dose vaccine in non-pregnant zebu cattle heifers (*Bos indicus*).

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MATERIALS AND METHODS

Animals and vaccination protocols: This randomized experimental trial was conducted at College of Veterinary Science and Animal Husbandry, UP Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidalaya Evam Go Anushandhan Sansthan (DUVASU), Mathura, India. Clinically healthy female calves (120) and heifers of 4 to 30 months of age, which were non-reactive in Rose Bengal Plate test (RBPT) and *Brucella* I-ELISA were enrolled for the present study. Before starting the experiment, all the animals were dewormed against internal parasites by recommended doses. Good management practices including feeding and watering were followed in the shed throughout the trial. All the 120 animals were assigned into 2 groups comprising 60 animals each, and each group was divided into 3 subgroups; vaccination was performed using different doses of vaccine (Table 1).

Administration of vaccine: The vaccine used in the study was manufactured and supplied by Indian Immunologicals Limited, Hyderabad, India having *Brucella abortus* S-19 strain. Each dose of *B. abortus* S-19 reduced dose vaccine contained 3×10^8 to 3×10^9 CFU. Each dose of standard vaccine contained $40\text{-}80\times10^9$ CFU/dose. At day 0, animals (groups 1A, 1B and group 2A, 2B) were administered with 2 ml of vaccine subcutaneously in the area drained by the left superficial cervical lymph node. Control animals (group

Table 1. Vaccination protocol for different groups and subgroups

Groups	Subgroups	Number of animals	Age of animals	Number of *B. abortus* S- 19 bacteria per dose
1	1A	40	4–12	3×10^8
	1B	10	months	to 3×10^9
				$40–80 \times 10^9$
	1C* (Control)	10		-
2	2A	40	13–30 months	3×10^8 to 3×10^9
	2B	10		$40–80 \times 10^9$
	2C* (Control)	10		-

*In control animals, same volume of sterile diluents was administered subcutaneously.

1C and 2C) were administered with the sterile diluent.

Sample collection: All vaccinated and control group cattle heifers were subjected to collection of blood samples on day 0, 21, 60, 90 post-vaccination in duplicate vide juglar venipunture with the help of vacutainers. For the estimation of cellular immune response blood samples were collected in heparinised vacutainers, while for humoral immune response in non-heparinised vacutainers.

Safety evaluation: After vaccination and placebo administration, all animals were observed daily for local reactions like pain, swelling, rashes, skin eruption, sloughing of mucous membrane, redness at administration site, etc. and/or systemic reactions (fever, loss of appetite/ anorexia, diarrhea and restlessness, etc.) and also for any serious adverse reactions.

Serological examination: Blood samples were examined for humoral response and cell mediated immunity (CMI). The humoral immune response was assessed by using acetone killed indirect ELISA (I-ELISA) as described by Colby *et al.* (2002). The actual OD values were obtained by subtracting the OD values of antigen coated well from non-antigen coated well. The zero day OD values of all the animals in the trial obtained from the ELISA test were used to calculate the cut-off values for analysis. The cut-off value was estimated by the following formula.

Cut-off Value= Mean + (3× Standard Deviation)

The samples below or equal to the cut-off value were considered to be seronegative. The samples above the cut-off value were considered to be seropositive (for sero-conversion).

Cell mediated immunity (CMI) was assessed on day 0 pre-vaccination and on day 21, 30, 60 and 90 post-vaccination employing interferon-gamma (IFN-γ) assays by using stimulated peripheral blood samples using killed *B. abortus* S-19 antigen. The interferon gamma response was assayed using kit. The IFN-γ assay results were expressed as stimulation index (SI) calculated as mean OD value of blood cell stimulated with specific antigen-mean blank at 450 nm / mean OD value of blood cell stimulated with PBS / media control-mean blank value at 450 nm. After arranging the zero day OD values by frequency distribution and considering the highly distributed values the cut-off was calculated from mean and standard deviation of those highly distributed values.

Statistical analysis: The results of I-ELISA and IFN-γ assays were analyzed by applying descriptive statistics and presented as mean ± standard error. Treatments were compared using one way analysis of variance (ANOVA) as per standard methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Safety evaluation: No local or systemic reactions were observed in the animals after vaccination or during follow up till the end of study. This is in agreement with previous studies (Beckett and MacDiarmid 1985, 1987), who also reported no local/systemic reaction with *Brucella abortus* strain-19 reduced dose vaccination. This supports the use of both the vaccine safely up to the age of 30 months.

Serological evaluations: As being intracellular pathogen, *Brucella* organism elicits both humoral and cell mediated immune response (CMI), so both responses were assessed.

Humoral immune response: Immune response to *Brucella* organism was measured with commonly existing serological tests but ELISA is more sensitive and specific to detect antibodies against *Brucella* in comparison to conventional methods (Gad *et al.* 1998), thus humoral response was evaluated with indirect ELISA. At the beginning of the study, all animals were found seronegative for *Brucella* antibodies on repeated examinations at the interval of 28 days. The humoral immune response based on I-ELISA is presented in Table 2. In group 1, the optical density (OD) values with reduced dose were comparable to the standard. However, the OD values were higher in standard dose on 60^{th} and 90^{th} day in comparison to reduced dose. There was no significant ($P<0.05$) difference between 2 groups. In group 2, the OD values with reduced dose were comparable to the standard on 21^{st} day post-vaccination, but were significantly ($P<0.05$) higher for reduced dose on 60^{th} and standard dose on 90^{th} day. All the control animals from group 1 remained seronegative throughout the study period. The control animals from group 2 showed some percentage positivity on different days that might be because of the lateral transmission from vaccinated animals as the animals were housed in the same shed (Table 3).

Both the groups revealed good antibody production (Table 2) in comparison to control group within 21 days post-vaccination and continued to increase 60 day post-vaccination. However, antibody response with reduced dose was comparatively lower than standard dose and it might be due to the lesser number of bacteria per dose of vaccine. There was no significant difference up to 60 days post vaccination in reduced and standard dose group and in young and adult animals. These findings were also in concurrence of earlier reports of having detectable antibodies (Beckett and MacDiarmid 1987, Schurig *et al.*

Table 2. Mean serum antibody response at different time intervals following vaccine administration

OD values	(Mean ± SE)	0 day	21st day	60th day	90th day
Group 1	1 A	0.255 ± 0.041	0.823 ± 0.047	0.937 ± 0.089	0.857 ± 0.089
(4 to 12 m	1 B	0.237 ± 0.033	0.729 ± 0.123	1.021 ± 0.184	1.039 ± 0.186
age group)	1 C	0.291 ± 0.027	0.107 ± 0.065	0.097 ± 0.014	0.341 ± 0.039
Group 2	2 A	0.274 ± 0.024	0.899 ± 0.041	1.199 ± 0.055	0.977 ± 0.082
(13 to 30 m	2 B (~12m)	0.277 ± 0.041	0.819 ± 0.084	0.841 ± 0.173	1.545 ± 0.135
age group)	2 C	0.364 ± 0.071	0.334 ± 0.126	0.281 ± 0.097	0.484 ± 0.056

A, Reduced dose; B, standard dose; C, control.

Table 3. Percentage of seroconversion in different groups

Group/DPV	0 day	21st day	60th day	90th day
Group 1A	0%	87.2%	74.4%	82.1%
Group 1B	0%	80.0%	70.0%	70.0%
Group 1C	0%	0%	0%	10%
Group 2A	0%	89.5%	94.7%	86.8%
Group 2B	0%	90.0%	70.0%	90.0%
Group 2C	0%	28.6%	14.3%	28.6%

2002, Caporale *et al.* 2010). Antibody response declined 90 day post-vaccination in both the age group with reduced dose while it persisted in standard dose groups. This might be of use as the antibody response in reduced dose group revealed good seroconversion which was comparable to the standard dose group animals (Table 2). Moreover, the results revealed that in reduced dose group it declined post 90 days of vaccination whereas, it sustained in standard dose vaccination group. This is in support of previous studies that reduced dose vaccination will have little hindrance in diagnosis of clinically positive animals (Beckett and MacDiarmid 1987). Moreover, decrease magnitude and duration of detectable immune response in immunized animals is required to eliminate infection sooner and thus lead to the removal of the antigenic stimulation for a high and protective response (Nicoletti and Winter 1990).

Cell mediated immune response (CMI): CMI response (Fig. 1) was higher in reduced dose group compared to the standard dose group. In group 1, CMI response in young animals vaccinated with reduced dose and standard dose have shown peak values on day 21 and later declined sharply on day 60 and 90, whereas, in control animals this variation was considered negligible. The reduced dose group showed higher values compared to that of standard dose group. In group 2, the peak response of CMI was observed on day 21 that declined sharply on day 60 and 90 of the study. This response was more prominent in animals vaccinated with reduced dose when compared to that of standard dose group. The control animals showed varied CMI response on these days.

For the assessment of quality of vaccination good CMI is indispensable to overcome intracellular bacteria. The stimulation of T-lymphocytes to have an immune response against invader pathogens particularly for intra cellular pathogens may prevent and limit reinfection for a period of

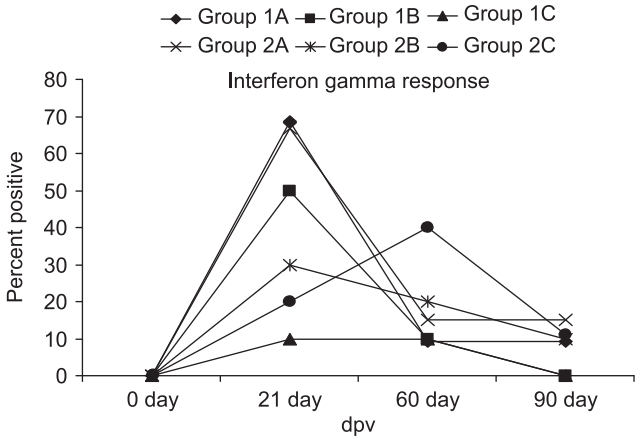

Fig. 1. Percent positivity for interferon gamma at different days after vaccination.

time (Bradley 2003) and this concept is also applied to have protection against brucellosis. Moreover, *in vitro* stimulation of bovine peripheral blood mono nuclear cells (PBMCs) with *Brucella* antigens leads to the production of bovine interferon gamma cytokine (BoINF-γ) that can be detected by ELISA (Meirerhoff *et al.* 2002). This Gamma interferon has advantages for detecting *Brucella* infection at the flock level as a complement to inconclusive results (Ferrer *et al.* 2004). The CMI response in both the age groups was assessed by gamma-interferon based assay and it revealed good response in both the age groups; and in both standard and reduced dose on day 21 post-vaccination. It declined sharply on day 60 and 90 which is a normal phenomenon. The gamma-interferon response was quite high in reduced dose group animals in comparison to animals vaccinated with the standard dose (Fig. 1). The high and durable gamma interferon production is expected in vaccinated animals as it as expected for the vaccination coverage (Tittarelli *et al.* 2009). Reduced dose animals produced better gamma interferon stimulation as compared to standard dose vaccine. Our findings are in the concurrence to the earlier findings that single dose of *Brucella abortus* strain-19 induced very little of T memory cells (Chukwu 1987), thus repeated vaccination or the booster are required to produce prolonged immunological memory. Similar to the findings in cattle (Alton *et al.* 1980), our study also revealed that the reduced dose vaccine is safe in both the age groups of animals, when administered subcutaneously with the production of good immune response comparable to the standard dose during

the study period of 90 days. Moreover, it also revealed shorter period of detectable serum antibodies and better gamma interferon stimulation.

In the countries like India, where test and slaughter policy cannot be implemented due to religious reasons, vaccination is the only other alternative to prevent and control brucellosis. However, the calf hood vaccination with existing *Brucella abortus* S-19 vaccines is limiting the use of vaccine. In such scenario, when *Brucella abortus* S-19 reduced dose vaccine is safe and effective in the young and non-pregnant zebu cattle heifers up to the age of 30 months with lesser chances of horizontal transmission and persistence antibodies, it can be vaccine of choice in zebu cattle for the prevention of brucellosis.

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