

## Safety and immunogenicity of *Brucella abortus* strain RB51 vaccine in cross bred cattle calves in India

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Safety and immunogenicity of *Brucella abortus* RB51 vaccine has been evaluated in an organized dairy farm in India. All the cattle (n=29) vaccinated with strain RB51 'responded' to the vaccine as demonstrated by iELISA using acetone killed strain RB51 antigen. The percentage responders at day 35, 60 and 90 post vaccination were 100%, 95% and 20%, respectively. Strain RB51 was able to elicit a good IFN- $\gamma$  response from vaccinated animals. The post-vaccination time point analysis indicated that the cumulative IFN- $\gamma$  response of whole blood from vaccinates stimulated with heat killed RB51 antigen was elicited in 80% of calves at 60 days post vaccination. Absence of strain RB51 in the secretions and excretion and lack of local or systemic reaction indicated the safety of the vaccine.

**Keywords:** Brucellosis, Cattle, RB51, Vaccine

Brucellosis is a zoonotic disease caused by several species of *Brucella* such as *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis* affecting cattle (white cattle and buffalo), goat, swine and sheep, respectively. High prevalence of brucellosis has been reported in India<sup>1</sup> resulting in severe economic loss. Currently, the vaccine available for prophylaxis is *B. abortus* S19 derived from smooth strain, which induces lipopolysaccharide (LPS) antibodies that interferes with the serological testing programs.

RB51 is a live attenuated vaccine derived from virulent rough *Brucella* strain 2308 grown in culture media containing variable concentrations of rifampin<sup>2</sup>. The strain is devoid of LPS O-side chains that are the dominant surface antigen of smooth brucellae and the

antigen detected by conventional brucellosis diagnostic tests<sup>3</sup>. Therefore, cattle vaccinated with RB51 do not seroconvert on conventional brucellosis serologic tests<sup>4</sup>. This makes the vaccine more appropriate than strain 19 for control and eradication programs. It has been reported that RB51 vaccine prevents abortion and infection in cattle under experimental and field conditions<sup>5-6</sup>. It is also the official live vaccine for cattle brucellosis in the U.S. and several other countries. The vaccine efficacy and stability of strain RB51 has been well demonstrated under laboratory as well as in field conditions<sup>7</sup> but limited information is available in countries like India where the disease is endemic and no published data is available for its field trial in an organized dairy farm.

Therefore, the present study was carried out to assess the safety and immunogenicity of *B. abortus* RB51 vaccine in Indian cross bred cattle up to 90 days post-vaccination.

A total 31 Jersey cross bred heifers, 4 to 12 months of age that were sero-negative in RBPT for *Brucella* antibodies were used. The animals were selected from Instructional Dairy Farm, Pantnagar. Two cattle calves inoculated with phosphate buffer saline served as negative controls. The animals were de-wormed and the general health status was monitored prior to vaccination.

*B. abortus* RB51 vaccine strain obtained and prepared under GMP conditions by Indian Immunologicals Limited, Hyderabad as per the protocol from VetTek, USA was used for vaccinating calves subcutaneously in the cervical region containing live strain RB51 at a concentration of  $1-3.4 \times 10^{10}$  colony forming units (CFU)/calf dose in 2 mL as recommended by Olsen *et al.*<sup>8</sup>

Blood samples were collected on 0, 21, 35, 60 and 90 days for evaluation of humoral and cell mediated immunity. Urine was collected on 0, 7, 14 and 21 days for bacteriological examination for the presence of *B. abortus*. Nasal, lacrymal and vaginal swabs were collected on 0, 7, 14 and 21 days for the same.

The humoral immune response to the vaccine was studied using an indirect ELISA as described by Colby *et al.*<sup>9</sup> Pre-vaccine sera collected on day 0 prior to vaccination and post-vaccination on day 21, 35, 60 and 90 were screened by indirect ELISA.

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Briefly, 600 nanogram of acetone killed RB51 was used as coating antigen. Blocking of unoccupied sites was done with 1% bovine gelatin (Sigma) in PBS-tween 20 (0.05%), pH 7.2. Test sera samples in dilution of 1: 200 were reacted with coating antigen in duplicate along with positive (1:100 dilution) and negative control sera (1:200 dilution). Protein A/G conjugate was used to bind IgG molecules in sera samples. Colour was developed using TMB/H<sub>2</sub>O<sub>2</sub> substrate solution and the change in intensity of the colour was determined at A<sub>450 nm</sub> by ELISA reader (Versaman, Molecular Devices).

Calculation of actual OD values with reference to departure in approach as mentioned by Colby *et al.*<sup>9</sup> Mean OD values obtained which are = or > mean  $\pm$  3 SD of negative control sera (day zero/pre-vaccine sera samples/sera samples from saline inoculated calves) were considered as positive responders. The cut-off for a positive antibody response to vaccine was established as optical density (OD) value of 0.7 at 450 nm derived from the mean OD of serum of saline inoculated controls at days zero and day 21  $\pm$  3 SD of this mean value.

CMI was assessed on day 0 pre-vaccination and on day 21, 35, 60 and 90 post-vaccination employing Interferon Gamma (IFN- $\gamma$ ) assay by stimulating peripheral whole blood samples using killed *B. abortus* antigen. The interferon gamma response was assayed using Bovigam kit (Prionics; USA).

Whole blood samples were collected from jugular vein using 18 gauge needle attached to a vacutainer coated with heparin (Beckton Dickinson). One ml of blood samples, each in duplicate, were stimulated with heat killed RB51 or *B. abortus* 544 antigens for strain RB51 vaccinated and saline inoculated control groups in 24 well cell culture plates (NUNC). Whole blood samples were also stimulated with pokeweed mitogen and PBS as positive and negative controls, respectively. The 24 well plates were incubated in humidified chamber at 37°C with 5% CO<sub>2</sub> for approximately 24 h; plasma was collected and stored at -70°C till processed. The IFN- $\gamma$  assay results were expressed as stimulation index (S.I.) [mean OD value of IFN- $\gamma$  from blood cells stimulated with specific antigen at 450 nm/mean OD value of IFN- $\gamma$  from blood cells stimulated with PBS control]. The cut-off S.I. value was derived as 4.85 for the RB51 vaccinated animals from the mean S.I. of plasma of saline inoculated controls at days 21 and 35  $\pm$  3 SD of this mean value.

The i-ELISA results were analyzed using One Way Analysis of Variance (ANOVA) and the IFN- $\gamma$  results were subjected to a 't' test for significance.

Nasal, lacrymal and vaginal swabs, collected using sterile swabs (Himedia), were dipped in *Brucella* broth (Himedia) transport medium supplemented with 2 $\times$  concentration of *Brucella* selective antibiotic (Himedia); sterile urine and heparinated blood samples transported on ice were inoculated on Rifampicin (250  $\mu$ g /mL) supplemented tryptose soya agar (TSA) with *Brucella* selective antibiotics. Inoculated plates were incubated at 37 °C under 5% CO<sub>2</sub> atmosphere for 5-7 days to check for the presence of strain RB51. The samples were collected as mentioned above on days 0, 7, 14 and 21 post-vaccination.

The shedding of vaccinal strain in urine were detected using bacteriological culture and examination. The samples were plated on antibiotic containing media to detect presence of *B. abortus* RB51.

There was no *Brucella* or RB51 vaccine strain recovered from nasal, lacrymal, vaginal swabs, urine and blood at day '0' in pre-vaccination samples from calves.

The initial screening of serum and blood samples at day zero (pre-vaccination) by RBPT, i-ELISA and IFN- $\gamma$  indicated that none of animals were positive by RBPT or i-ELISA but seven animals showed stimulation indices above IFN- $\gamma$  cut-off values and therefore not considered for analysis.

There were no local or systemic reactions observed. All the animals under trial were monitored for the presence of *Brucella abortus* RB51 in the body secretions and excretions. The animals were examined for bacterial secretion in nasal, lacrymal, urine and vaginal swabs collected on 0, 7, 14 and 21 days post vaccination. The vaccine strain RB51 could not be recovered from any sample at all time point of collection.

The serum antibody response induced by RB51 as determined by i-ELISA titer indicated that by day 21 post vaccination 100 % of the cattle responded to the strain RB51. The percentage responders at day 35, 60 and 90 post vaccination were 100, 95 and 20% respectively.

None of serum samples were positive by RBPT in RB51 vaccinated calves from day 21 to 90 post-vaccination.

The highest percentage of IFN- $\gamma$  responders (80%) was observed at 60 days post-vaccination when

stimulated with RB51 antigen that differed in response up on stimulation with *B. abortus* 544. However, the initial IFN- $\gamma$  response on day 21 post-vaccination to RB51 (53.3%) and 544 (46.6%) antigens did not differ significantly (Fig. 1).

*B. abortus* RB51<sup>TM</sup> vaccine was developed by Veterinary Technologies Corporation (VetTek), Virginia, USA and has been used in many countries of the world<sup>10-14</sup>. Indian Immunologicals Limited (IIL), Hyderabad in collaboration with Veterinary Technologies Corporation, USA has manufactured *B. abortus* RB51<sup>TM</sup> vaccine under GMP conditions and the present clinical trial was conducted to study the humoral and cell mediated immune response in cattle vaccinated with RB51 vaccine. For safety, the shedding of vaccinal strain in urine using bacteriological culture and examination was done. Interpretation of RBPT of RB-51 vaccinates and its comparison with that of *Brucella* positive sera was also included.

In the present study, as a vaccine, strain RB51 appeared to be safe and was capable of eliciting antibody and IFN- $\gamma$  response in cattle calves. 100% of the cattle vaccinated with strain RB51 'responded' to the vaccine as demonstrated by iELISA using acetone killed strain RB51 antigen. The percentage responders at day 35, 60 and 90 post vaccination were 100, 95 and 20%, respectively. Strain RB51 was able to elicit a good IFN- $\gamma$  response from vaccinated animals. The post-vaccination time point analysis indicated that the cumulative IFN- $\gamma$  response of whole blood from vaccinates stimulated with heat killed RB51 antigen was elicited in 80% of calves at 60 days post-vaccination.

There were no local or systemic reactions observed. This indicates that the vaccine does not

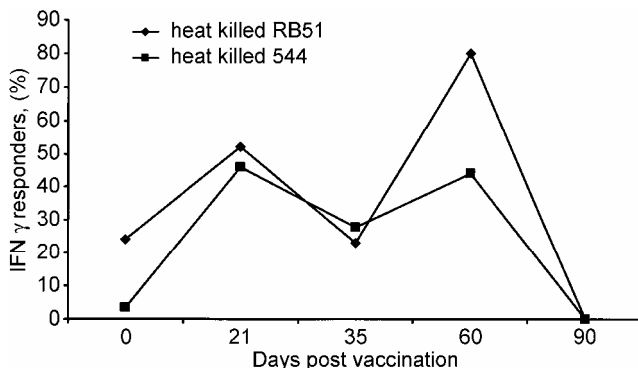


Fig. 1—IFN- $\gamma$  response of peripheral blood samples from cattle calves vaccinated with strain RB51.

induce any local or systemic reaction when inoculated in to animals. All the samples examined for bacterial presence could not yield any bacteria showing that the vaccinated animals will not be a source of infection to other animals.

It was also demonstrated that, RBPT can be used as tool for distinguishing between strain RB51 vaccinated animals and naturally infected or S19 vaccinated animals, as none of the strain RB51 vaccinated animals were positive by RBPT from day 21 to 90 post-vaccination. This again confirms the use of vaccine more appropriately for control and eradication programs that rely on serologic testing and removal of positive animals. RB51 strain has been the official vaccine used in the USA since 1996 for the prevention of brucellosis in cattle; hence this study will encourage for use of the strain RB51 in the vaccination programme in India where the disease is endemic.

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