



## Sero-monitoring of calf hood vaccination under brucellosis control program in selected states of India during 2016-18

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Brucellosis is a contagious zoonotic bacterial disease affecting livestock and humans worldwide. It is caused by *Brucella abortus* in cattle and buffaloes. The clinical signs include abortions, birth of weak calves, reduced milk yield in females and orchitis, epididymitis, and reduced fertility in bulls (Carvalho *et al.* 2010). The disease is rare or absent in some of the developed countries but it remains a major disease in developing and underdeveloped countries, causing considerable economic loss and public health complications. In India, loss due to brucellosis in cattle and buffalo is 95.6% of the total median losses of US \$3.4 billion occurring in livestock population (Singh *et al.* 2015).

In the country, recently observed increase in disease prevalence is attributed to intensive farming practices, introduction of unscreened infected new animals into the clean farms, uncontrolled movement of animals, unscreened bulls used for semen collection and natural service, unhygienic handling of abortions, etc. (Smits and Kadri 2005). Control of brucellosis is possible by addressing these risk factors along with mass vaccination, test and slaughter, and maintenance of sanitary and hygienic conditions.

The most widely used control measure is vaccination with *B. abortus* S19 vaccine in calves of 4-8 months age. The same vaccine in adult animals poses difficulties in differentiating infected from vaccinated animals on serological testing or may cause abortion in pregnant animals. To overcome these problems, *B. abortus* RB51 vaccine is being used, having less effectiveness during high prevalence and rifampicin resistance; thus making *B. abortus* S19, the most preferred vaccine in cattle and buffaloes. Hence, Department of Animal Husbandry and Dairying (DAH&D), the Government of India introduced Brucellosis-Control Program (B-CP) in 2012 to vaccinate

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calves of 4-8 months with the *B. abortus* S19 vaccine.

Although the program was planned for the entire country, few states took a lead in implementing vaccination from 2014 onwards. Post-vaccination sero-monitoring is an important parameter for evaluating vaccination coverage. Thus, this study aimed to evaluate S19 *Brucella* vaccination program in different states of India during 2016-18 by assessing *Brucella* antibodies post-vaccination and looking at further possible strategies for enhancing the success of the program.

DAHD, Government of India has implemented B-CP in the country. During the period 2016-18, a cross-sectional study was carried out in assessing the *B. abortus* S19 vaccination implemented in the country. Nine states of India, viz. Chhattisgarh, Gujarat, Himachal Pradesh, Karnataka, Maharashtra, Nagaland, Rajasthan, Tamil Nadu, and Telangana were selected for the study, where calf hood vaccination for brucellosis was undertaken. A sample size of 246 was determined for each state considering 80% sero-prevalence (expected percentage of antibodies in vaccinated calves), 5% precision, and 95% confidence interval (Cochran 1977). State Animal Husbandry Departments were requested to collect serum samples of the required sample numbers between 21 to 240 days of post-vaccination (DPV) from female calves wherever vaccination with *B. abortus* S19 vaccine was carried out. The states were instructed to collect 2-3 mL of blood using vacutainers without anticoagulant from the jugular vein of female calves. The serum samples separated from clots, centrifuged at 5000 rpm for 5 min were dispatched to ICAR-NIVEDI under the cold chain. Serum samples were screened by in-house developed protein G based indirect enzyme-linked immunosorbent assay (iELISA) (Indian Patent No. 335659) with a sensitivity of 95% and specificity of 96%, respectively. The coating antigen is smooth lipopolysaccharide (sLPS) from *B. abortus* S99 strain and the cut-off per cent positivity (PP) value for a sample to be considered negative is below 55% and positive is 55% and above.

Confidence interval and chi-squared analyses between the states and between various DPV were calculated using R Studio (R Core Team 2020). The graph was represented by GraphPad Prism version 7.00 and the map was created using QGIS version 3.16 software.

In this study, 52.25% positivity for *Brucella* antibodies was recorded in 2,687 serum samples of calves vaccinated with *B. abortus* S19 in the nine states. Overall antibody response for *Brucella* vaccination was extremely good in the states of Telangana, Himachal Pradesh, and Maharashtra; moderate in Tamil Nadu, Gujarat, Nagaland and Chhattisgarh states; and poor in Karnataka and Rajasthan (Fig. 1 and Table 1). Chi-square analyses between the states as well as various DPV were significant, which implied an association between the states and also between various DPVs. Despite the fixed sample size for each state was set, few states provided more samples than requested and Tamil Nadu state failed to provide requisite samples and hence wider confidence interval of 16.32%

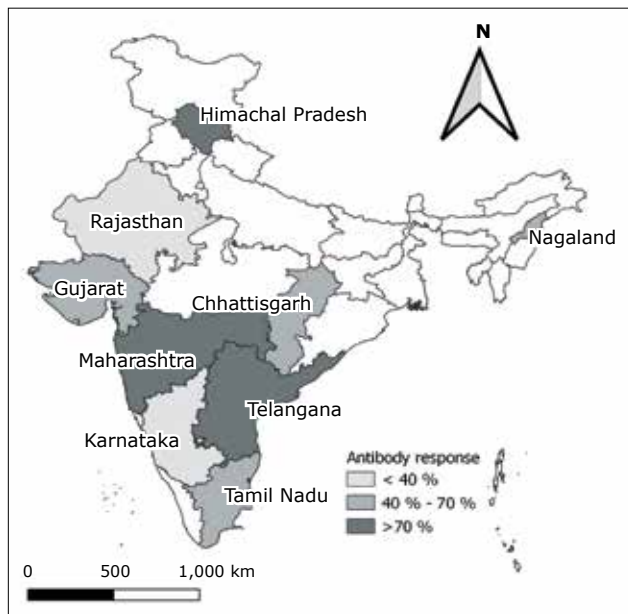


Fig. 1. Spatial representation of *Brucella* S19 vaccine antibody response (%) in female calves over the surveyed states of India (Telangana state represented in this map is before bifurcation of Andhra Pradesh state into Andhra Pradesh and Telangana).

was observed. The samples with no DPV data showed coverage of 35.26% indicating that many of the samples might have been collected beyond 45 DPV.

Antibody response was high during 21-45 DPV and declined gradually as the number of DPVs increased. Increased Per cent Positivity (PP) values in ELISA after 14 DPV, reaching peak during 28 DPV and declining thereafter at 120 DPV in an experimental S19 vaccination trial conducted in buffalo calves has been similarly reported (Shome *et al.* 2020). In this study, there was a gradual reduction in the number of positives from the group DPV21\_45 to DPV181\_240 with decreased PP values of <50% in ELISA from DPV61\_120. Although the presence of antibodies can be detected up to many months in calves vaccinated with *Brucella* vaccine, as per this study, it is preferable to collect sera between 45 to 60 DPV for assessing *Brucella* antibodies. Antibody response against *Brucella* vaccination over various DPV is presented in Table 2 and state-wise antibody responses over various DPV are represented in Fig. 2.

The objective of vaccination is to reduce the number of susceptible animals in the population and the success

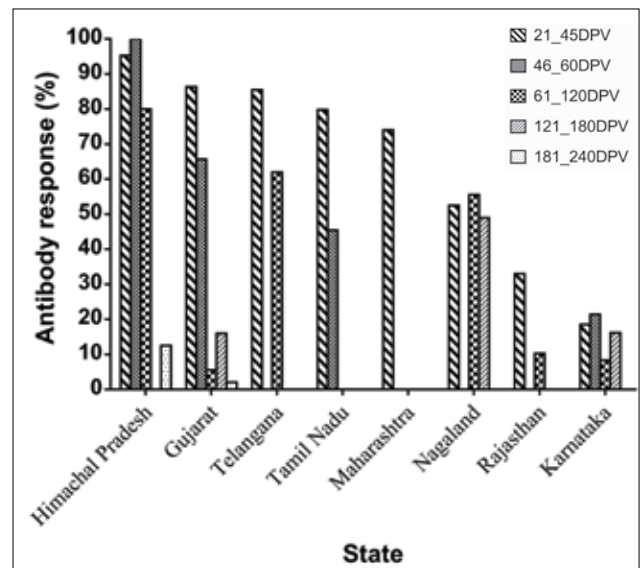


Fig. 2. State-wise *Brucella* S19 vaccine antibody response in female calves.

Table 1. State-wise per cent positivity for *Brucella* antibodies in female calves

State	Total number of samples	Number of samples showing <i>Brucella</i> antibodies	Per cent positivity	95 % CI	$\chi^2$
Chhattisgarh	226	102	45.13	38.57-51.87	592.64 (P<0.0001)
Gujarat	289	153	52.94	47.01-58.79	
Himachal Pradesh	268	217	80.97	75.64-85.39	
Karnataka	352	72	20.45	16.44-25.13	
Maharashtra	254	188	74.02	68.09-79.20	
Nagaland	291	153	52.58	46.67-58.41	
Rajasthan	464	106	22.84	19.16-26.99	
Tamil Nadu	148	87	58.78	50.39-66.71	
Telangana	395	326	82.53	78.34-86.07	
Total	2687	1404	52.25	50.34-54.15	

Table 2. DPV wise percent positivity for *Brucella* antibodies in female calves

DPV	Total number of samples	Number of samples showing <i>Brucella</i> antibodies	Per cent positivity	95 % CI	$\chi^2$ (DPVNA not included)
DPV21_45	1260	924	73.33	70.78-75.74	388.60 (P<0.0001)
DPV46_60	139	78	56.12	47.45-64.43	
DPV61_120	380	123	32.37	27.74-37.36	
DPV121_180	185	44	23.78	17.98-30.70	
DPV181_240	65	3	4.62	1.20-13.76	
DPVNA	658	232	35.26	31.63-39.06	
Total	2687	1404	52.25	50.34-54.15	

depends mostly on the effectiveness of the vaccine and its coverage in the target population (Dorneles *et al.* 2017). For mass vaccination to be effective for brucellosis, the recommended coverage should reach at least 80% (Zinsstag *et al.* 2005). In this study, the antibody response in the vaccinated calves was above 70% in three states, namely Telangana, Himachal Pradesh and Maharashtra, out of nine studied states. Although there may be various factors for reduced antibody response in other states, an increase in antibody response is of paramount importance for ensuring effective protection and control against brucellosis. Nyerere *et al.* (2020) suggested mass vaccination combined with environmental hygiene and personal protection to attain brucellosis-free equilibrium in cattle for more than six years. Environmental contamination should be addressed effectively as the transmission rate for the host cattle from the environment may be greater than infectious cattle (Zhang 2014). As most livestock farmers are ill-informed about the zoonotic risk, creating awareness on economic importance of the disease and benefit of the brucellosis control program is the most important requirement for the success of the program. Simultaneous efforts are needed for controlling brucellosis in sheep, goats, and pigs (Avila-Granados *et al.* 2019). Strengthening information dissemination and improved veterinary and public health surveillance, and the establishment of diagnostic facilities will add value to the control program (Jiang *et al.* 2020).

Recently, to give more impetus to brucellosis control, the Government of India relaunched a megaproject entitled “National Animal Disease Control Program” (NADCP) for the control of Foot and Mouth Disease (FMD) and brucellosis. Thus, this pilot survey will help to understand the vaccination coverage countrywide through mass vaccination to control brucellosis under NADCP.

#### SUMMARY

The study aimed to evaluate the post-vaccination antibody response in sera of 4-8 months old female calves vaccinated with *Brucella* S19 vaccine under Brucellosis-Control Program (B-CP) initiated by Department of Animal Husbandry and Dairying (DAH&D), Government of India during 2016-18. The antibody response was extremely good in three states [Telangana (82.53%), Himachal Pradesh (80.97%) and Maharashtra (74.02%)]. Higher antibody response was observed during 21-

45 days post-vaccination (DPV) which indicated this period as appropriate for sampling to assess the antibody response. The knowledge acquired with respect to the post-vaccination sero-monitoring in this study will help the ongoing brucellosis control program under the flagship program of Government of India launched in 2019 as National Animal Disease Control Program (NADCP).

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