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# Safety of *Brucella abortus* Strain 19 Reduced Dose (s/c) Vaccine in Pregnant Cattle

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## ABSTRACT

Live *Brucella abortus* strain 19 attenuated vaccine (standard dose vaccine) is the most widely used against bovine brucellosis. Even though it induces reasonable protection against *Brucella abortus*, persistent serological responses interfere with brucellosis diagnosis. To minimize residual antibody titers and to prevent occasional persistent vaccinal infection, a reduced dose vaccine with a CFU per dose of  $3 \times 10^8$  to  $3 \times 10^9$  organisms subcutaneously to calves is advocated. However, it is anticipated that some animals may develop persistent antibody titres and may abort. Hence, a study was conducted to evaluate safety, incidence of vaccine induced abortions and isolate excretion of vaccinal organism during parturition after vaccination with *Brucella abortus* S19 reduced dose vaccine by subcutaneous route in pregnant cattle. One hundred and six healthy pregnant cattle at different stages of gestation were vaccinated with *Brucella abortus* S19 reduced dose subcutaneous vaccine. All the animals were followed upto parturition. No abortion was observed in any of the vaccinated animals. At normal parturition, the amniotic fluid samples were collected aseptically from 20 vaccinated cows, and the samples were bacteriologically analysed for the presence of the vaccine strain. All these samples were tested negative for *Brucella abortus* in microbiological culture examination. It can be concluded that *Brucella abortus* S19 reduced dose subcutaneous route vaccine can be safely used in pregnant cattle for prevention of brucellosis.

**Keywords** Brucellosis, Pregnant cattle, *Brucella abortus* S19 vaccine, Abortion

## INTRODUCTION

Many countries have controlled/eliminated bovine brucellosis by implementing three basic principles: (i) test and slaughter, (ii) maintaining sanitary conditions, and (iii) vaccination of animals [1]. But in India, the policy of test and slaughter of cows cannot be implemented due to religious sentiments and the positive-testing animals cannot be culled for economic considerations. Maintenance of sanitary conditions is a difficult task and is not followed on majority of the dairy farms. Mass vaccination is the primary way of brucellosis control in livestock. This should be combined with other measures such as methods that limit the spread of the pathogen, allow identification of animals and herds, and increase community participation in the control program. The most widely used vaccine for the prevention of brucellosis in cattle is the *Brucella abortus* S19 vaccine, which remains the reference vaccine to which any other vaccines are compared. *Brucella abortus* S19 vaccine induces good immunity to moderate challenge by virulent organisms. Vaccinating with S19 vaccine is considered to give long-lasting immunity and subsequent doses are not recommended. The beneficial effects of Strain 19 have been proven in a large number of experiments and cattle herds. The post vaccinal titre problems have been studied extensively. The vaccine has been administered by numerous routes and differing doses:

*Brucella abortus* S19 vaccine is used as a live avirulent/attenuated vaccine and is normally given to female calves aged between 3 and 6 months or (4-8 months) as a single subcutaneous dose of  $5-8 \times 10^{10}$  viable organisms (calfhood vaccination) [2]. Calfhood vaccination has several advantages such as; it is effective method of control of abortion in the herd and there will be reduction in the reactor losses in herds and also less number of tests to eliminate brucellosis from infected herds [3]. In some countries, S19 has been used to vaccinate adult cattle to

increase the immunity in herds with high risk of brucellosis [4]. However, persistence of antibodies increases with age of vaccination and vaccinating adult cattle with S19 often leads to the production of persistent antibody titers which makes interpretation of subsequent serological tests difficult [5,6]. Some animals may abort and excrete vaccine strain in milk. A reduced dose of vaccine that contains  $3 \times 10^8$  to  $3 \times 10^9$  organisms can be administered subcutaneously to adult cattle [2]. The side effects are much lesser than that of full dose vaccination. The reduced dose is much safer than full dose adult vaccination. Alton et al. [7] found that a much reduced dose of Strain 19 ( $3 \times 10^8$ ) was effective and reduced the persistence of titres and vaccine infections in pregnant cows. The immunity provided by reduced dose of vaccine is as solid as given by full dose. In the present investigation, the number of pregnant cattle considered were more than all the earlier studies carried out.

The present study was undertaken to evaluate safety of *Brucella abortus* S19 reduced dose (S/C) vaccine in pregnant cattle to study any vaccine-induced abortions in pregnant animals and to evaluate any excretion of vaccinal organisms during normal parturition.

## MATERIALS AND METHOD

One hundred and six healthy pregnant cattle, seronegative for *Brucella* antibodies, were enrolled in the study. Positive control did not keep in the present as higher dose of vaccine induces abortions in pregnant animals. The animals were at different stages of gestation at the time of vaccination with *Brucella abortus* S19 reduced dose subcutaneous vaccine as shown in Table 1. All the animals received 2 ml dose of *Brucella abortus* S19 vaccine containing  $3 \times 10^8$  to  $3 \times 10^9$  viable organisms subcutaneously in the mid neck region. The animals were observed for occurrence of any abortions during the study period. At normal parturition, amniotic fluid samples were collected randomly from 20 vaccinated cows and the samples were bacteriologically analysed for the presence of the *Brucella abortus* S19 vaccine strain using Potato infusion agar (PIA) media according to Alton et al. [8,9] (Techniques for the Brucellosis Laboratory; OIE Terrestrial Manual 2009). The plates were observed for colony formation at each dilution (neat,  $10^{-1}$ ,  $10^{-2}$ ) and in the event of any colony formation; it was identified by grams staining and observed for the morphology of *Brucella abortus* S19 under microscope.

**Table 1:** Stage of pregnancy of the enrolled animals at the time of *Brucella abortus* S19 reduced dose S/C vaccination

Number of pregnant animals vaccinated	Stage of gestation
54	Early gestation
32	Mid gestation
34	Late gestation

## RESULTS AND DISCUSSION

All the animals were healthy throughout the experimental period. No abortion was observed in any of the vaccinated animals. The bacteriological test results of amniotic fluids collected from the vaccinated animals are shown in Table 2. All these samples were tested negative for *Brucella abortus* in microbiological culture examination. In some of the amniotic fluid samples, microbial contamination was observed.

**Table 2:** Excretion of *Brucella* organisms and microbial contaminants identified in amniotic fluid of cattle vaccinated with *Brucella abortus* S19 reduced dose S/C vaccine

Viable count & Purity by plate method with <i>Brucella</i> selective media											
S. No.	Stage of gestation	Animal		Neat			10 <sup>-1</sup> (Dilution)			10 <sup>-2</sup> (Dilution)	
		Tag No.	Brucella organisms	Microbial contaminants	Brucella organisms	Microbial contaminants		Brucella organisms	Microbial contaminants		
				Contaminants	Viable count	Contaminants	Viable count	Contaminants	Viable count		

1	Early gestation	1302	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
2		1328	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
3		1323	Negative	<i>Staphylococcus sciuri</i>	176	Negative	<i>Staphylococcus sciuri</i>	35	Negative	<i>Staphylococcus sciuri</i>	4
5	Mid gestation	1318	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
6		1303	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
7		1305	Negative	1) <i>Staphylococcus sciuri</i> 2) <i>Erysipelothrix rhusiopathiae</i>	80	Negative	1) <i>Staphylococcus sciuri</i> 2) <i>Erysipelothrix rhusiopathiae</i>	3	Negative	---	Nil
8		1317	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
9		1311	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
10		1313	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
11	1319	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--	
12	Late gestation	1324	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
13		1316	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
14		1326	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
15		1325	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
16	Late gestation	1329	Negative	1) <i>Erysipelothrix rhusiopathiae</i> 2) <i>Bacillus species</i>	92	Negative	1) <i>Erysipelothrix rhusiopathiae</i> 2) <i>Bacillus species</i>	14	Negative	1) <i>Erysipelothrix rhusiopathiae</i> 2) <i>Bacillus species</i>	6
17		1321	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
18		1330	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
19		1327	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--
20		1314	Negative	Nil	--	Negative	Nil	--	Negative	Nil	--

The present study was undertaken to evaluate safety of *Brucella abortus* S19 reduced dose vaccine administered through subcutaneous route and to find out if vaccination would cause abortions in pregnant animals and to study if there would be any excretion of *Brucella abortus* organisms during normal parturition. Animals enrolled in different stages of pregnancy were observed during their entire gestation period till their natural parturition or abortion. Throughout the study period none of the vaccinated pregnant animals aborted and all the pregnant animals had natural parturition. This is in agreement with the findings of Pinna et al. [10], who reported that there were no abortions in cattle vaccinated with S19 vaccine and concluded that the S19 reduced dose vaccine was safe as it did not induce abortion and effective in abortion prevention in study animals. Similar observations was made by Alton et al. [7] who reported very negligible rate of abortions (0.56%) and concluded that pregnant cows could be effectively vaccinated by the subcutaneous administration of a reduced dose of strain 19 vaccine. However, these observations were contradictory to that of Beckett and MacDiarmid [11] who reported abortions in 8 out of 307 (2.6%) three to five months pregnant animals which were vaccinated with a reduced dose of *Brucella abortus* strain 19 vaccine. The dose of  $5 \times 10^8$  CFU S19 in pregnant cattle will cause abortions, but the rate of vaccine abortions is <1% [12].

Amniotic fluid samples of twenty randomly selected experimental animals tested negative for presence of *Brucella* organisms according to bacteriological diagnostic tests. The sterile plate of Potato infusion agar (PIA) was used as the media for processing of amniotic fluid samples. This procedure was adopted from Teaching Manual on bacterial vaccines and immuno-diagnostics, IVRI that states that inoculation of bacterial suspension onto potato infusion agar (PIA) plate with 2% glycerin will produce only pure and smooth culture of *Brucella abortus*. In this study, *Brucella abortus* S19 strain organisms could not be isolated from any of the amniotic fluid samples collected from the vaccinated animals during parturition. This is contrary to the findings of Alton et al. [7], who reported excretion of Strain 19 organisms after parturition and premature calving with heavy infection of the placenta and foetus with Strain 19 in both cases.

The results of the present study support the use of reduced dose S19 vaccine given through subcutaneous route in pregnant cattle under field conditions in India and confirm the results found by other authors. In Indian scenario, where no other policy except vaccination is possible and practicable for the prevention and control of brucellosis, a mass vaccination strategy using *Brucella abortus* S19 reduced dose vaccine by subcutaneous route covering all groups of female cattle including calves, adult and pregnant animals is desirable.

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