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Milk excretion study of *Brucella abortus* S-19 reduced dose vaccine in lactating cattle and buffaloes

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Abstract
Present study is aimed to determine the excretion of *Brucella abortus* organisms in milk of vaccinated lactating cattle and buffaloes. All experimental lactating animals in early and mid-stage of lactation were vaccinated with *Brucella abortus* S-19 reduced dose vaccine through subcutaneous route. After vaccination daily milk samples from all the animals i.e. 10 cattle and 10 buffaloes were collected in sterile sample collection vials from day 1 till day 30 for bacteriological analysis. Milk collected from all vaccinated animals was not used for human consumption and was discarded throughout the study period. Sterile Potato infusion agar (PIA) media plate was used for detection of *Brucella abortus* organisms. For identification of contaminants BDBBL Crystal ID (Becton, Dickinson and Company) was used. All the experimental lactating animals were serologically negative for brucellosis at the time of enrollment. Milk samples from the animals that were collected and tested up to 30 days, were found negative for *Brucella abortus* in microbiological culture, although in all of the milk samples, contamination was observed.

Keywords: Bovine brucellosis, *Brucella abortus* S19 vaccine, Milk excretion

Introduction
Bacterial organisms of genus *Brucella* that are pathogenic for wide variety of animals and humans beings. (McMahan, 1944) [3]. in animals, abortion is the most obvious manifestation. Infections may also cause stillbirths or weak calves, retained placentas and reduced milk yield (Nicoletti, 2013) [5]. Brucellosis is readily transmissible to humans, causing acute febrile illness and undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculo–skeletal, cardiovascular and central nervous systems. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory or conjunctival routes but ingestion of dairy products constitutes the main risk to the general public where the disease is endemic (OIE, 2009) [6, 8].

Public health importance of brucellosis is much related to the infected animal species from which human transmission occurs. The most rational approach for preventing human brucellosis is the control and elimination of the diseases in animal reservoir and health education of the public working at high risk area (Dubie *et al*., 2014) [1].

Brucellosis is an occupational hazard. It is primarily a disease of animals transmitted directly or indirectly to man. Dairy workers, shepherds, veterinarians, abattoir workers and animal husbandry personnel are particularly at risk. It constitutes an uncontrolled public health problem in many developing countries (Madkour, 1989) [2]. Infected cows shed Brucella in their milk and this is key in its transmission to calves. In dairies, milking is another mode of transmission that must be taken into account because the bacteria are highly likely to be transmitted from cow to cow if the same teat cups are used for milking (Samartino, 2003) [12].

The mammary gland and regional lymph nodes can also be infected and bacteria can be excreted in milk (OIE, 2010) [8]. Female calves can also be infected during birth when passing through infected the birth canal or by suckling colostrum or milk from infected cows. While most of these calves rid themselves of Brucella a small percentage may continue to be infected until adulthood remaining negative to diagnostic serological tests but aborting during their first pregnancy. Such animals pose a serious threat to brucellosis control and eradication (Ter Huurne et al, 1993) [13].

Brucella abortus S-19 has remained the cornerstone for most Brucellosis eradication program since 1940. Calfhood vaccination with S-19 vaccine lead to progressive reduction in cases of brucellosis globally. Vaccination is usually performed on young female calves between three to eight months of age. Vaccination of adult cattle with S19 low dosage ($3 \times 10^8 - 3 \times 10^9$) CFU was also successfully employed in infected herds. In general, after vaccination S19

Calfhood does not persist in the reproductive tracts of mature heifers and does not cause abortion in these animals. Nonetheless, even with markedly infrequent occurrence, some cattle remain chronically infected and may abort and excrete vaccine strain in the milk. Almost all cows vaccinated with strain 19 do not shed the organisms in milk for any length of period. It is shed in milk for a day or two in a few animals (less than 1%).

The present study was undertaken to evaluate safety of *Brucella abortus* S-19 reduced dose (S/C) vaccine in lactating animals and to determine if any excretion of *Brucella abortus* organisms in milk occurs in vaccinated lactating animals.

Material and Methods

10 lactating cattle and 10 lactating buffaloes, seronegative for Brucella antibodies, were enrolled in the study. The animals were at first and second stage of lactation at the time of vaccination with *Brucella abortus* S19 reduced dose subcutaneous vaccine (Table 2 and 3). All the animals received 2 ml dose of 3 x 10^8 to 3 x 10^9 viable *Brucella abortus* S19 vaccine subcutaneously in the mid neck region. The animals were observed for excretion of *Brucella abortus* organisms in milk throughout the study period of 30 days. Daily milk samples from all the animals (10 cows + 10 buffaloes) were collected in sterile sample collection vials from day 1 till day 30 for bacteriological analysis. Aseptic milk collection was ensured by teat dip and udder spray with disinfectant solution before milk collection. Milk was not used for human consumption and was discarded from all vaccinated animals throughout the study period.

Sterile Potato infusion agar (PIA) media plate was used in this study (OIE, 2012 and Ryan, 1967) [7, 11]. One vial of Brucella selective supplement (suspension of 1 vial of Brucella selective supplement in 50% methanol) was added in 500 ml of Brucella media. The plates were observed for any colony formed at each dilution and if present, identified by Gram's staining and observed for the morphology of *Brucella* under microscope.

Method for identification of contaminants

BDBBL Crystal ID: (Becton, Dickinson and Company)

The isolated colony from the inoculum was suspended in the tube of BBL crystal respective inoculum fluid. As per the procedure of BDBBL crystal further it was incubated for 18-20 hours at 35-37 ºC for Enteric/Non Fermentor, 18-24 hours at 35-37 ºC for gram positive and 4.0 hours at 35-37 ºC for anaerobic organisms.

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

Number of lactating animals vaccinated	Stage of lactation
4	Early lactation (cattle)
6	Mid lactation (cattle)
7	Early lactation (buffaloes)
3	Mid lactation (buffaloes)

Results and Discussion

All the lactating animals i.e. 10 cattle and 10 buffaloes were serologically negative for brucellosis at the enrollment. They were administered with *Brucella abortus* S19 reduced dose vaccine through subcutaneous route. Milk samples from all the experimental animals that were collected and tested up to 30 days. The milk sample test showed negative for *Brucella abortus* in microbiological culture, although in all the milk samples, contamination was observed. The details of contaminants observed in the milk of cattle and buffaloes is given in table 2 and 3 respectively.

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

Bruvax Plus (Brucella abortus S19 reduced dose)-Milk excretion study-(Route of Administration:S/C wth 2ml)													
Cow (ILFC, College of Veterinary Science, Rajendranagar, Hyderabad)													
Stage of lactation →			Early lactation				Mid lactation						Remarks
Animal Tag No: →			216	232	1333	1334	1339	1366	37	40	115	122	
S. No	Sample details	Day of collection	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	Viable Count	
1	Cow milk	Day1	5	3	3	4	4	6	6	3	4	3	The following microbial contaminants observed 1) *Brevundimonas diminuta* 2) *Kytococcus sedentarius* 3) *Agrobacterium tumifaciens* 4) *Strepto coccus* vestibularis 5) *Micrococcus luteus* 6) *Lactococcus rafinolactis*. No *Brucella organisms* were found.
2	Cow milk	Day2	6	5	4	3	3	4	3	3	5	6	
3	Cow milk	Day3	5	4	3	3	4	6	5	4	4	3	
4	Cow milk	Day4	3	4	4	4	6	5	4	3	5	4	
5	Cow milk	Day5	6	4	5	3	6	4	4	4	5	3	
6	Cow milk	Day6	3	4	3	4	3	3	3	4	5	4	
7	Cow milk	Day7	5	4	4	3	5	6	3	3	6	3	
8	Cow milk	Day8	3	4	3	6	5	4	3	5	5	3	
9	Cow milk	Day9	3	3	5	4	4	5	5	3	3	6	
10	Cow milk	Day10	4	4	4	5	5	4	3	4	5	5	
11	Cow milk	Day11	3	5	4	4	4	5	3	5	6	4	
12	Cow milk	Day12	4	6	5	3	3	4	4	4	4	4	
13	Cow milk	Day13	5	5	6	3	6	3	3	5	4	5	
14	Cow milk	Day14	6	4	5	4	6	4	3	4	5	3	
15	Cow milk	Day15	5	4	4	3	3	3	5	3	6	3	
16	Cow milk	Day16	3	3	5	4	3	3	5	4	3	6	
17	Cow milk	Day17	5	3	4	6	5	3	3	5	3	4	
18	Cow milk	Day18	4	3	3	3	4	5	3	4	5	4	
19	Cow milk	Day19	6	4	6	4	3	5	6	3	3	3	
20	Cow milk	Day20	5	5	3	5	4	3	6	5	3	3	
21	Cow milk	Day21	6	3	5	4	5	4	6	4	5	3	
22	Cow milk	Day22	6	3	4	4	4	3	6	5	3	6	
23	Cow milk	Day23	6	4	3	4	5	3	4	4	4	4	

24	Cow milk	Day24	3	3	6	5	4	3	6	5	4	6	
25	Cow milk	Day25	4	6	5	5	5	3	4	3	4	3	
26	Cow milk	Day26	5	5	3	5	3	6	4	4	4	3	
27	Cow milk	Day27	4	4	6	5	4	3	5	3	5	3	
28	Cow milk	Day28	5	4	5	3	5	3	4	6	5	3	
29	Cow milk	Day29	4	5	3	5	3	6	6	4	5	3	
30	Cow milk	Day30	5	3	5	6	5	5	3	6	4	5	

Table 3: Excretion of *Brucella* organisms and microbial contaminants identified in milk of buffaloes vaccinated with *Brucella abortus* S19 reduced dose S/C vaccine

Bruvax Plus (Brucella abortus S19 reduced dose) - Milk excretion study - (Route of Administration : S/C wth 2ml)												
Buffalo (ILFC,College of Veterinary Science,Rajendranagar,Hyderabad)												
Stage of lactation →			**Early lactation**							**Mid lactation**			**Remarks**
Animal Tag No: →			**430**	**497**	**457**	**498**	**519**	**530**	**435**	**509**	**505**	**513**	
S. No	**Sample details**	**Day of collection**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	**Viable Count**	
1	Buffalo milk	Day1	4	3	5	3	6	3	4	6	4	4	The following microbial contaminants observed 1) *Brevundimonas diminuta* 2) *Micrococcus luteus* 3) *Pseudomonas fluroescens* 4) *Lactococcus raffinolactis* 5) *Sphingomonas paucimobilis*. No *Brucella organisms* were detected.
2	Buffalo milk	Day2	3	6	4	3	4	3	3	4	5	3	
3	Buffalo milk	Day3	5	6	3	5	3	4	3	6	3	5	
4	Buffalo milk	Day4	3	5	5	3	4	5	3	4	4	3	
5	Buffalo milk	Day5	6	3	4	3	5	3	4	3	4	3	
6	Buffalo milk	Day6	5	3	4	3	4	3	6	3	5	4	
7	Buffalo milk	Day7	3	6	3	5	6	4	3	4	5	3	
8	Buffalo milk	Day8	4	4	5	4	4	3	4	3	6	4	
9	Buffalo milk	Day9	6	3	5	4	3	5	3	3	4	3	
10	Buffalo milk	Day10	4	3	3	4	5	3	6	5	5	3	
11	Buffalo milk	Day11	3	4	3	5	6	3	5	4	5	3	
12	Buffalo milk	Day12	5	3	3	5	5	4	3	4	3	5	
13	Buffalo milk	Day13	3	4	3	4	3	3	4	5	3	5	
14	Buffalo milk	Day14	4	5	3	6	3	5	3	4	3	3	
15	Buffalo milk	Day15	4	3	4	3	3	4	3	5	5	6	
16	Buffalo milk	Day16	5	6	5	4	3	3	3	4	3	6	
17	Buffalo milk	Day17	3	4	5	4	4	5	6	3	4	3	
18	Buffalo milk	Day18	3	3	4	4	5	4	5	3	4	4	
19	Buffalo milk	Day19	6	3	4	3	5	5	3	6	5	6	
20	Buffalo milk	Day20	3	4	5	4	4	4	4	5	3	3	
21	Buffalo milk	Day21	3	6	5	5	4	3	3	4	3	5	
22	Buffalo milk	Day22	3	5	3	5	3	4	4	3	4	3	
23	Buffalo milk	Day23	5	4	3	3	4	3	4	3	4	3	
24	Buffalo milk	Day24	4	3	4	5	4	3	3	3	3	4	
25	Buffalo milk	Day25	4	5	3	4	4	5	5	3	4	4	
26	Buffalo milk	Day26	4	3	6	6	3	6	5	5	4	3	
27	Buffalo milk	Day27	5	3	4	3	4	5	4	4	4	5	
28	Buffalo milk	Day28	6	3	3	4	3	3	4	3	6	5	
29	Buffalo milk	Day29	3	3	5	3	4	4	3	4	3	4	
30	Buffalo milk	Day30	3	4	5	4	3	4	5	4	5	3	

In the present study, *Brucella abortus* S19 strain was not isolated from any of the milk samples of vaccinated animals (Table 2 and 3). This is in agreement with the findings of Pacheco *et al.* (2012) [9] who found all the milk samples negative for *Brucella spp.* in microbiological culture. Although the milk samples were negative for *Brucella abortus* organisms, microbial contamination was observed in all the milk samples. Microbial contamination included pathogens like *Micrococcus luteus, Pseudomonas fluorescens, Brevundimonas diminuta, Kytococcus sedentarius, Agrobacterium tumefaciens, Streptococcus vestibularis, Sphingomonas paucimobilis, Lactococcus raffinolactis* bacteria tend to be released in very large numbers and create elevated counts. The findings were similar to the findings of Murphy (1997) [4] who found rapid growth of bacterial species under warm conditions to produce high colonies count. The contamination found in the milk samples might generally occur from three main sources; within the udder, exterior to the udder and from the surface of milk handling and storage equipments and the surrounding air, feed, soil, feces and grass (Parekh and Subhash, 2008; Torkar and Teger, 2008) [10, 14].

It can be concluded for the present study that the *Brucella abortus* S19 reduced dose subcutaneous vaccine is safe when administered to lactating cattle and buffaloes and will not cause excretion of *Brucella* organisms in milk.

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