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SHORT COMMUNICATION

Milk Ring Test for spot identification of *Brucella abortus* infection in single cow herds

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ABSTRACT

In this study, milk samples were collected from 109 dairy cows to detect antibodies against *Brucella* (*B.*) using Milk Ring Test (MRT). Overall, 18.35% (n=20/109) of the milk samples were positive by MRT. The cows were divided into three groups based on lactation number *viz.*, 1st, 2nd to 4th and ≥5th lactations; the prevalence of brucellosis in the groups were found to be 0.92% (n=1/109), 15.60% (n=17/109) and 1.83% (n=2/109), respectively. Considering simplicity and cost effectiveness, the MRT can be used for the preliminary screening of *B. abortus* infection especially in single cow herds.

Keywords:
Brucellosis, cows, diagnosis, milk ring test, zoonosis

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INTRODUCTION

Brucellosis is primarily a disease of dairy cows causing economic losses to the livelihoods of many farmers around the world. In recent years, the cases of bovine brucellosis have been increased in India, possibly due to increased trade and rapid movement of livestock. Despite of various preventive and control measures being followed in India, there is still a high potential for the transmission and spread of *B. abortus* due to its widespread prevalence (Renukaradhya et al., 2002). Further, the risk of acquiring infection from unpasteurized milk is a major cause as raw milk is traditionally consumed in India where the hygienic aspects are not always sufficiently considered (Lingathurai and Vellathurai, 2010).

A number of serological tests are widely used for the diagnosis of brucellosis because infected cattle may or may not produce all antibody types in detectable levels. The MRT, first described in Germany by Fleischhauer (1937), is used as a routine periodic test for brucellosis free herds and for identifying infected herds. The MRT is an agglutination test conducted on fresh milk collected from dairy cattle, but it does not work on pasteurized or homogenized milk (Fleischhauer, 1937). The MRT, which detects IgM and IgA antibodies bound to fat globules, may have wide acceptability as it is cost effective, easy to perform and can cover a large population in a short time (Cadmus et al., 2008).

Besides, the MRT is considered as an ideal method for detecting infected herds and for diagnosis of brucellosis in individual animals (Noriello, 2004) though it is known from very early studies that false positive reactions may occur in colostrum or milk at the end of the lactation period and milk from cows suffering from a hormonal disorder or mastitis (Morgan, 1967; Bercovich and Moerman, 1979). Considering the advantages of MRT such as simplicity, wide acceptability and cost effectiveness, the present study was designed to envisage the usefulness of MRT for a preliminary screening of *B. abortus* infection in single cow herds as it involved non-invasive sampling.

Table 1: Results of the MRT of the animals based on the number of lactation

Lactation group	Positive		Suspected		Negative	
	No.	%	No.	%	No.	%
1st	1	0.92	6	5.50	-	-
2nd - 4th	17	15.60	58	53.21	18	16.52
5th & above	2	1.83	5	4.59	2	1.83
Total (n=109)	20	18.35	69	63.3	20	18.35

MATERIALS AND METHOD

Sample collection and handling: A total of 109 lactating cows of different age groups were sampled, collecting 10 ml milk pooled from 4 quarters. The breed and stage of lactation of each cow were recorded. To get the more reliable results, the milk samples obtained from the animals were kept refrigerated at 4°C overnight prior to examination by MRT (Alton et al., 1988). The milk samples were mixed well to ensure an even distribution of the milk cream.

In the present study, the animals were grouped into three groups. First group comprised of cows with 1st lactation, second group having cows with 2nd to 4th lactations and third group with cows in ≥5th lactations.

Milk Ring Test (MRT) for identifying infected cows: The milk ring test is the most practical method for locating infected dairy animals and for surveillance of brucellosis-free herds. The test was performed by adding 30 µl (0.03 ml) of *B. abortus* Bang Ring Antigen (hematoxylin-stained antigen manufactured by the State Biological Laboratory, Institute of Veterinary Preventive Medicine, Ranipet, India). The height of the milk column in the tube was kept up to 25 mm. The milk (antigen) mixtures were incubated at 37°C for 1 h, together with positive and negative control samples. Agglutinated *Brucella* cells were picked up by fat globules as they rose, forming a dark cream layer on the top of the sample. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. The test was considered negative if the color of the underlying milk exceeded that of the cream layer and when the cream layer was normal. Samples were read as negative, 1+, 2+, 3+ and 4+ depending on the intensity of color in the cream layer.

The criteria of reactions for whole milk was kept as recommended by Genset et al. (1956) with a slight modification in the color of cream layer following the test as this reagent was a haematoxylin-stained antigen while the stain used by Genset et al. (1956) was tetrazolium blue. The criteria of reactions are given below:

Negative reaction (-): cream ring white, skim milk fraction blue white;
Suspicious reaction (1+): cream ring pale pink but less colored than the skim milk fraction;
Suspicious reaction (2+): the pink color of the cream ring equal to that of the skim milk fraction;
Positive reaction (3+): color of cream ring deeper pink than that of the skim milk fraction;
Positive reaction (4+): cream ring pink, skim milk fraction white.

RESULTS AND DISCUSSION

The prevalence of brucellosis in the 1st lactation group was 0.92% (n=1/109). The group comprising of 2nd to 4th lactations was mostly prevalent (15.60%; n=17/109) with brucellosis as compared to other groups (Table 1). On the other hand, 1.83% (n=2/109) milk samples of 5th and above lactation group were found positive to brucellosis. In the first lactation group, the lower prevalence of brucellosis could be attributed to resistance of sexually immature cattle which become susceptible to the disease with age, or passive immunization of calves through colostrum of their infected dams (Mohammed et al., 2011). Multiparous cows showed increased prevalence of brucellosis which was supported by Matope et al. (2011). Sukumar et al. (2012) in a similar observation found that maximum percentage (12.5%) of animals were positive for brucellosis at 7-years age group followed by 6 (11.1%), 8 (11.1%) and 5 (9.8%) years age groups; while, no prevalence was found in young animals (≤4-years age). This was a possible reflection that aged animals had more chances of exposure to the bacteria and contracting disease. In an earlier observation by Rezaei et al. (2010) in Iran, the MRT showed 14% positive reaction for *B. abortus* taking many age groups into consideration unlike this study.

The milk ring test is an inexpensive test for the surveillance of dairy herds for brucellosis because milk and whey samples can be obtained easily and they have been widely used for testing herds or individual animals for antibodies. The stronger the MRT reaction is, the more likely is the fact that *B. abortus* can be isolated by culture (Leech et al., 1964). Though no attempt was made to isolate the *B. abortus* in the milk it

is a matter of great significance as lactogenic excretion of *B. abortus* just after parturition in cows can lead to infection in humans where the practice of consuming raw milk is quite common. The MRT reported to have a sensitivity of 89% (Nicoletti, 1966; Hunter and Allen, 1972). Recently, Salman et al. (2012) found similar levels of sensitivity and specificity for MRT which were 85% and 95%, respectively. The test was similar to the protocol employed in this study and was preferred due its simplicity to identify *B. abortus* infection particularly in single cow herd situations. However, the MRT has also been reported for distinct disadvantages including false positive results in milk samples collected shortly after parturition, near end of lactation, mastitic cows or vaccinated animals (Macmillan, 1990). This study was done with care by avoiding all these situations.

CONCLUSIONS

The milk ring test is a simple procedure for day to day screening of *B. abortus* in single cow herds. No conclusion could be drawn on the sensitivity of the test as the true status of the animals was unknown. Therefore, it is suggested that other confirmatory tests, like the milk ELISA, are to be used in conjunction for establishing disease status. The risk of infection to other animals and humans (zoonosis) is also to be considered in such positives cases for which the MRT is an ideal eye opener. It is, however, obvious that the MRT is the first line of screening test for brucellosis particularly in single cow herds.

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