

## Milk Ring Test as Ready Aid to Diagnose Bovine Brucellosis in Lactating Cows of Tamil Nadu, India

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### Abstract

In this cross sectional study 483 milk samples were collected from 483 lactating cows from selected districts of Tamil Nadu to detect antibodies against *Brucella* using Milk Ring Test (MRT). Overall, 4.35 per cent of milk samples were positive by MRT. The lactating cows were divided into 1st, 2nd to 4th and  $\geq 5$ th lactation and the prevalence of *Brucella* were 2.94, 3.06 and 6.48 per cent respectively. Based on husbandry practices, the highest prevalence was recorded in unorganized farm sector (8.21%) followed by organized farm (3.84%) and single cow herds (3.63%). MRT can be used as a spot screening test. Presence of antibodies with their prevalence in organized and unorganized farms warrants a systematic preventive strategy that is used to control brucellosis.

**Keywords:** Brucellosis; Cow; MRT; Farms

### Introduction

Brucellosis is one of the highly contagious reproductive diseases in dairy animals and highly prevalent among bovine population in India [1]. Generally, *Brucella* infection in animals leads to severe economic impact by causing abortion, infertility, retention of placenta, still birth and calf loss in animals [2]. In cattle brucellosis is mostly caused by *Brucella abortus*, other causative agents like *B. melitensis* and *B. suis* have also been reported [3].

Brucellosis is considered as the second most zoonotic disease next to Rabies [4]. The main source of infection in Indian population was dairy products. The milk of infected sheep, goats or cattle may contain large numbers of viable organisms, which become concentrated in products such as yogurt, paneer and cheese [5]. In dairy animals, the organism localizes in the supra-mammary lymph nodes and mammary glands of 80% of infected animals, and these continue to excrete the pathogen in milk throughout their lives acting as carriers but intermittently [6].

Thus effective identification, control and eradication of bovine brucellosis are a global concern and can be achieved only by early, reliable and accurate diagnosis and vaccination. But brucellosis is a complicated disease in terms of diagnosis because of non-pathognomonic nature of infection and the clinical diagnosis cannot be generalized to all age groups, sex, breed and physiological status especially in non-pregnant animals, heifers and bulls. As a result, many cases remain undiagnosed and cause outbreaks in organized dairy farms, there by spreading the disease to other animals and humans [7].

A number of serological tests are widely used for the diagnosis of bovine brucellosis and still now there is no single test that reliably confirms brucellosis status except culture. Because, all infected animals may or may not produce all antibody types in detectable levels and culture techniques is considered as gold standard technique in *Brucella* diagnosis. However, culture of *Brucella* is most difficult one due to its

fastidious nature [3] as well as high zoonotic potential being a laboratory acquired zoonoses.

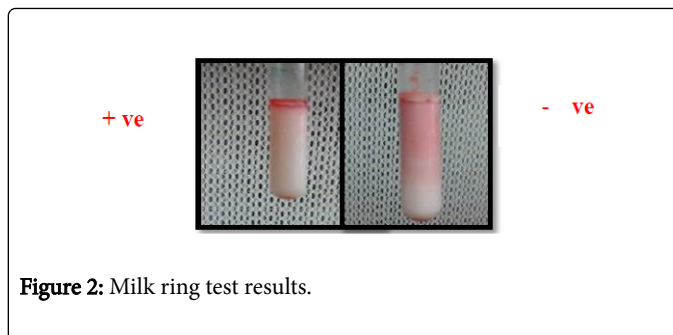
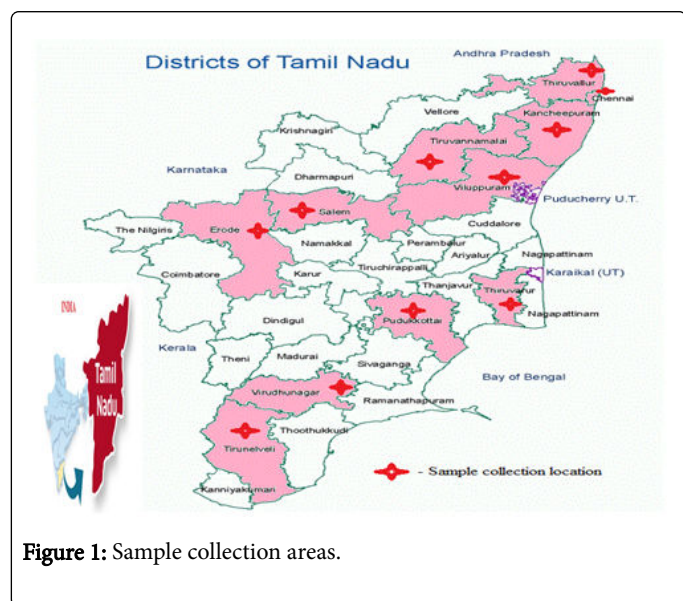
Other than serum, *Brucella* antibodies are also excreted in milk and milk being a non-invasive sample, sampling a large population can be covered in a short time. MRT was first described by Fleischhauer [8] in German and it is often used as a herd test to know the prevalence of *Brucella* infection and for screening the herd. However, MRT can also be used to test individual milk samples but, it may give false-positive results shortly after parturition, near the end of lactation and when mastitis is present [9]. MRT which mainly detects IgM and IgA antibodies against *Brucella* infection in fresh milk [10]. The present study was focused to assess the prevalence of *Brucella* antibodies in milk samples using MRT as a spot test.

### Materials and Methods

The present study was conducted in certain districts of Tamil Nadu (n=483), viz., Erode (n=82), Salem (n=56), Kancheepuram (n=68), Tiruvallur (n=33), Tiruvannamalai (n=41), Viluppuram (n=45), Thiruvarur (n=39), Pudukkottai (n=45), Virudhunagar (n=31), Tirunelveli (n=37) and Chennai (n=6) (Figure 1). Based on farm-wise the sampling frame were Organized government farms (University Research Farm (n=6) and Post Graduate Research Institute for Animal Sciences (n=25) of TANUVAS, Chennai), private farms (Erode, Tiruvannamalai and Virudhunagar) (n=21), Unorganized farms (n=73) and single cow herds (n=358) to assess the current status of *Brucella* infection.

Lactating animals were selected randomly from the study area with exclusion of mastitis affected animals, animals which non-vaccinated against brucellosis and immediately calved animals to avoid colostrum that could cause false positive reactions in MRT. *Brucella abortus* Bang ring test antigen was obtained from the Institute of Veterinary Preventive Medicine (I.V.P.M), Ranipet. The antigen was stored at 4°C until use. The MRT was performed, as per guidelines of OIE, 2009 [3]. MRT was performed on individual milk samples. Antigen and milk

samples were brought to a room temperature prior to performing the test. About 50 µl of antigen was added to 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes then were incubated at 37°C for one hour together with positive and negative working standards. A strongly positive reaction was indicated by formation of dark pink ring above a white milk column. The test was considered to be negative if there was uniform pink colour for the milk column and cream layer (Figure 2).



## Results

In this present study the prevalence of *Brucella* infection in milk sample were 4.35 per cent by MRT. The prevalence in lactating cows was divided into 1<sup>st</sup>, 2<sup>nd</sup> to 4<sup>th</sup> and ≥5<sup>th</sup> lactation and was 2.94, 3.06 and 6.48 per cent respectively (Table 1).

Lactation stages	Total screened	No. of positive	MRT	% positivity
1st lactation	102	3		2.94%
2nd to 4th lactation	196	6		3.06%
≥5th lactation	185	12		6.48%
Total	483	21		4.35%

**Table 1:** Lactation stage-wise prevalence of bovine brucellosis by MRT

Husbandry Practices	Total No. screened	No. of MRT positive	% positivity	
Organized	Government (URF and PGRIAS of TANUVAS)*	31	1	3.22%
	Private farms	21	1	4.76%
Total organized farm	52	2	3.84%	
Unorganized farm	73	6	8.21%	
Single cow herds	358	13	3.63%	
Total	483	21	4.35%	

**Table 2:** Husbandry practices-wise prevalence of bovine brucellosis by MRT. (\*URF - University Research Farm, PGRIAS - Post Graduate Research Institute in Animal Sciences, TANUVAS - Tamil Nadu Veterinary and Animal Sciences University)

Based on husbandry practices-wise seroprevalence of brucellosis was high in unorganized farms (8.21%) followed by organized farms (3.84%) and single cow herds (3.63%) (Table 2).

## Discussion

### Prevalence of brucellosis by MRT

In this present study the prevalence of *Brucella* infection in milk sample were 4.35 per cent. These findings were concurred with the reports of Kang'ethe et al. [11] (3.9%) whereas Chand et al. [12] (7.9%), Rehman et al. [13] (11.4%), Zowghi et al. [14] (25.21%), Mahato et al. [15] (35.82%) and Junaidu et al. [16] (25.25%) reported higher prevalence than present study. The variation in prevalence of

brucellosis by MRT might be due to variation in the sampling, interpretation by individual and clinical conditions of animals. It is to be known that mastitis milk, colostrum milk and last stage of milk may give false positive reactions over MRT (OIE, 2009) [3]. Hence mastitis and colostrum milk were excluded in the present study which might be reason for lower prevalence.

### Lactation stage-wise prevalence of bovine brucellosis by MRT

In this study, lactating cows were divided into 1<sup>st</sup>, 2<sup>nd</sup> to 4<sup>th</sup> and ≥5<sup>th</sup> lactation and the prevalence were 2.94, 3.06 and 6.48 per cent respectively (Table 1). The highest prevalence was recorded in ≥5<sup>th</sup> lactation groups which were already documented by Botha et al.

[17-20]. Pleuriparous cows showed increased prevalence of brucellosis which was in accordance with the reports showed Matope et al. [21]. The prevalence of *Brucella* infection in dairy cows was found to be higher as age advanced in this study. Aulakh et al. [22] reported that, the disease prevalence was found less common in young animals which were attributed due to resistance of sexually immature cattle to infection, which become susceptible to disease with age [23], or passive immunization of calves through colostrum of their infected dams. Similar results have been reported by various authors [18,24]. Contradictory to present study, Mohamand et al. [25] found more prevalence in 2<sup>nd</sup> to 4<sup>th</sup> lactation group than ≥5<sup>th</sup> lactation groups which might be due to sampling size, demography, disease epidemiology and clinical condition of individual animals.

### Husbandry practices-wise prevalence of bovine brucellosis by MRT

Based on husbandry practices seroprevalence of brucellosis was high in unorganized farms (8.21%) followed by organized farms (3.84%) and single cow herds (3.63%) (Table 2). There are various researchers who reported management wise prevalence of bovine brucellosis in India. In this study, the results coincided with the findings of Isloor et al. [26-28] showing that a higher prevalence of brucellosis was observed in organized farms than in single cow herds. This may be attributed to the spread of infection that is quick, easily spread and mainly due to the improper screening, surveillance and monitoring. Organized private farm had regular screening tests conducted contributing to for less prevalence of brucellosis. Shome et al. [29] documented that purchase of animals without prior screening for brucellosis, lack of awareness and lack of routine milk testing were found as other potential risk factors for transmission.

Comparatively single cow herds were lesser in prevalence than farms because of lesser number of animal preventing interactions, cutting down transmission routes due to the sparse animal density [30]. High prevalence in unorganized sector may be attributed due to improper screening of brucellosis, sample size, natural service of animals with same bull which can act as a focus of infection to other animals, unhygienic conditions [31] at house hold levels and improper disposal of aborted materials.

The present cross sectional study concluded that, 4.35 per cent of milk samples were found positive for brucellosis in this study area. No conclusion can be drawn about sensitivity of MRT because we do not know the exact true status of the animals tested. However, it is stable spot on test, readily feasible for field conditions and further confirmation can be achieved by culture and Enzyme Linked Immunosorbent Assay techniques. Regular and systematic screening of brucellosis is essential to control brucellosis and on the bigger picture, its zoonotic importance.

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