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Milk ring test to screen brucellosis in dairy animals of Jabalpur

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Abstract

Total 34 herds including 14 cow herd, 12 buffalo herds and 8 goat herds which are suspected for brucellosis infection in the animals on the basis of herd history like having abortions in animals and repeat breeder, were screened in this study. Out of 14 cow herds 5 herds, 12 buffalo herds 8 herds, and 8 goat herds 1 herd was screened as suspected. Prevalence rate among the cow herds was 35.0% (5/14), buffalo herds 66.66% (8/12) and 37.5% (1/8). This study indicates prevalence of brucellosis in dairy animals although further investigation using more sensitive methods with individual animal sampling is required.

Keywords: Milk ring test (MRT), herd screening, cattle, buffalo, goat

Introduction

Brucellosis one of important zoonotic diseases. This disease is of economic importance in terms of animals affected having abortion, decreased milk production, reproductive problems, infertility in males etc. The milk ring test (MRT) is cheap and rapid test which can be use for screening of herds positive for brucellosis. This test detects IgM and IgA antibodies in milk bound to fat globules. Pooled milk samples are used for this purpose. Using this test herd can be classified as negative or suspected, for confirmation other test like RBPT (Rose Bengal plate test), ELISA or PCR using specific primers can be used to identify the infected animals and for epidemiological study purpose. The test was first described by Fleischhauer in 1937. This test can be use for initial screening of herds and it can serves as alternative to serum based test which require collection of blood samples, as collection of milk is quite easy especially in large animals.

Materials and Methods

Total 34 herds were screened in this study. Milk samples were collected from different dairy animal herds for the screening purpose. Samples were pooled from each 20 animals one pooled sample (50 ml) was collected. Samples were kept at 4 °C and processed as soon as possible to laboratory. Before conducting the test milk samples were mixed properly. Test were performed as per standard procedure described by Genset *et al.* (1956) [3], by adding 30 µl of *B. abortus* Bang ring antigen procured from ICAR-IVRI, in small sized glass test tubes. The height of milk columns were kept 25 mm. Milk mixed with MRT antigen were incubated at 37 °C for 1 hr. one positive and one negative control samples were also kept. In positive samples there is formation of dark pink ring above the milk column, where as in negative samples cream layer remains white and underlying milk column has intense pink color than cream layer.

Table 1: Details of samples collected

Animal	No of Herds	No of Animals
Cow	14	243
Buffalo	12	1315
Goat	08	188
Total	34	1746

Results and Discussion

Total 34 herd which include, 14 cow herd, 12 buffalo herds and 8 goat herds were screened which are suspected for brucellosis infection in the animals on the basis of herd history.

These herds are having abortions in animals and repeat breeder. Out of 14 cow herds 5 herds, 12 buffalo herds 8 herds, and 8 goat herds 1 herd was screened as suspected. Prevalence rate among the cow herds was 35.0%, buffalo herds 66.66% and 37.5%.

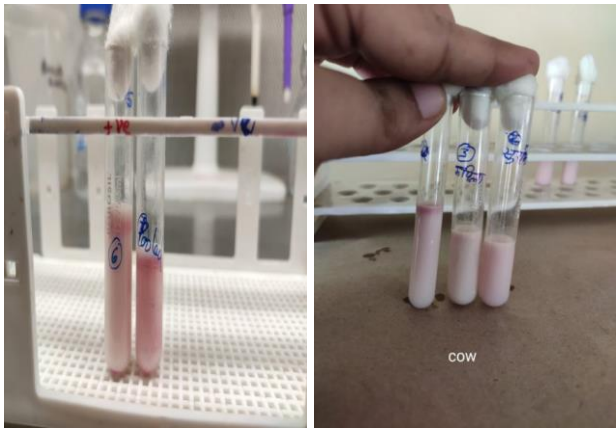


Fig 1: MRT for cow milk

Tube 1: Individual animal milk (Positive);
 Tube 2 and 3: Pooled milk from positive herd milk
 Tube 4 and 5: Individual animal milk (Negative) for brucellosis

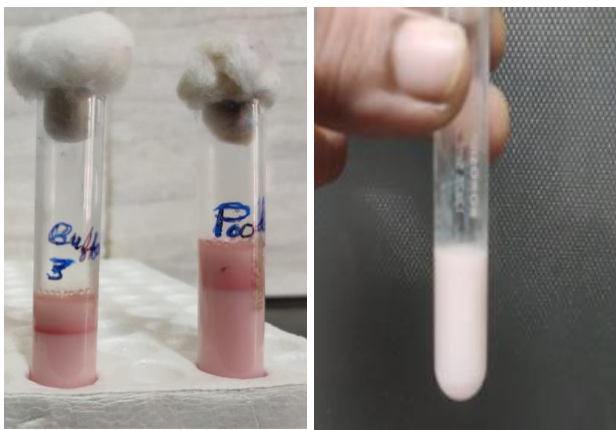


Fig 2: MRT for Buffalo milk

Tube 1: Individual animal milk (Positive);
 Tube 02: Pooled milk (Positive);
 Tube 3: Individual animal milk (Negative) for brucellosis

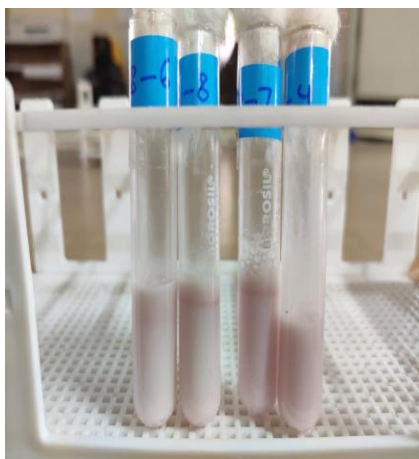
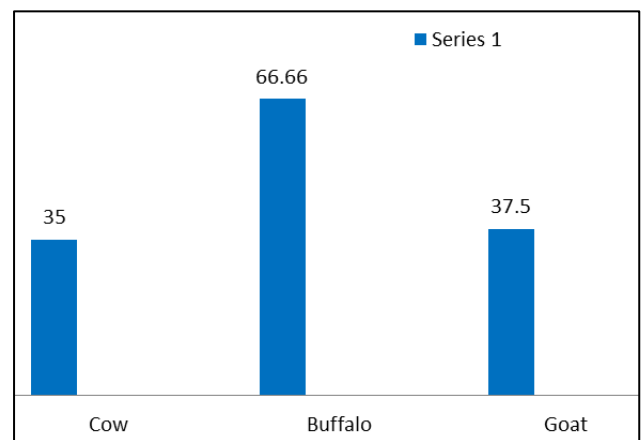


Fig 3 MRT for Goat milk

Tube 1 and 4 –Pooled milk sample negative for brucellosis;
 Tube 2 and 3 – Pooled milk sample positive for brucellosis

The test is prescribed by OIE for cow milk (OIE, 2009) [7]. this test is not work on pasteurized or homogenized milk. This test can also be use in individual animals also (Noriello, 2004) [6] in this study also test was applied on some samples using individual animal milk from suspected herds but in this individual animal milk testing it shows less sensitivity and the ring obtained in some cases was faint in comparison to pooled milk samples. In this study 1746 animal, consisting of 1315 buffaloes, 243 cows and 188 goats from 34 herds were screened. On the basis of result of this study prevalence of brucellosis was high in buffaloes followed by cow and goat population. High prevalence among the buffalo population may be because most of the buffalo are reared under semi intensive and intensive farming system. High incidence was reported in herds maintained in intensive or semi intensive system. Low incidence among the goat population may be due to as goats in this area are reared by small farmers under extensive system for meat purpose and they sell these animals every year and most animals are not reaching breeding age and due to extensive rearing system the load of pathogen in the shed or pan is less. Another reason for low prevalence in goat population may be antigen used in this test is *B. abortus* strain while goats are most susceptible for *B. melitensis*. In similar study done by Singh *et al.* (2020) [11] in dairy animals in gaushalas of Braj egiion of Uttar Pradesh 9.92% (13/131) prevalence was reported. in another study conducted by Dalal *et al.* (2017) [1] in and around Jaipur and overall 21.73 % milk samples showed positive MRT. In another study in Vindhya region of Madhya Pradesh, 4.58% milk samples of cows were found positive with milk ring test (Singh *et al.*, 2016)[10]. Kumar *et al.* (2016) [4] screened milk samples in Tamil Nadu and 4.35% of milk samples were found positive for brucellosis using MRT. Shome *et al.* (2015)[9] in a detailed study found overall positivity of 2.55% in pooled milk. Mohamand *et al.* (2014) [5] have reported overall, 18.35% (n=20/109) of the milk samples were positive by MRT in Chennai. Dubey and Mathur (1980) [2] found prevalence of brucellosis 4.51% in cows and 3.48% in buffalo milk samples in the Ajmer and Tonk districts of Rajasthan by the milk ring test. In various earlier studies, there has been a variation in prevalence of brucellosis by MRT this indicated the regional variation in the prevalence of brucellosis in bovines.



Prevalence of brucellosis with MRT test in cow, buffalo and goat herds

Single MRT have 65% probability to detect reactors in cow herd (Roepke and Stiles, 1970) [8]. More frequently the herd

tested using this test more helpful it will be as MRT has good sensitivity in early stage of infection. Small sample of pooled milk from 20-25 animals is used for test. Main limitation of this test is, in large population false negative results may be obtained due to dilution; to overcome this large sample volume can be use. In recently vaccinated animals, milk samples having colostrum or mastitis or late lactation milk also leads to false positive results. So care should be taken. As the test is less sensitive so at least test should be repeated frequently at least one herd should be tested in every 2-3 months. This test is not work on pasteurized or homogenized milk. Over all prevalence among the herds in Jabalpur region was very high as per this study and this is a matter of concern from public health point of view. High prevalence reported in this study may be also due to as the herd selected in this study was having history of abortions and other reproductive problems and most animals are reared under intensive farming system. For further validation of result and for identification of infected animals more sensitive and specific test like RBPT, ELISA, PCR etc. should be applied.

Conclusion

MRT is relatively inexpensive and rapid test for screening of brucellosis in herd and individual animals although false positive reaction may be there but if correlated with history of herd and animal along with simultaneous use of other sensitive test for individual animal screening this test can be useful for control and prevention of spread of brucellosis among animal herds.

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