

# Surveillance of Infertility and Abortions due to Brucellosis in Cattle Farms in Bidar District of Karnataka

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## ABSTRACT

The present study was aimed to conduct both syndromic and active surveillance of brucellosis in major reproductive disorders in 200 cattle including repeat breeding (53), anestrus (34), retention of fetal membranes (RFM, 64), and abortions with retained fetal membranes (49). For syndromic surveillance, pooled milk samples of 5 tehsils/farms and 200 individual milk samples (40 per farm) were analyzed using the milk ring test (MRT). Active surveillance involved testing blood samples using the Rose Bengal plate test (RBPT) and indirect ELISA (iELISA). Among the reproductive issues observed in the study area, RFM was the most prevalent accounting for 32% (64 cases) followed by repeat breeding (26.5%), abortion with retained placenta (24.5%) and anestrus (17%). Syndromic surveillance through MRT identified brucellosis in 16.32% (8/49) of abortion cases with retained placenta, 6.25% (4/64) of RFM cases, 5.6% (3/53) of repeat breeder animals and 2.94% (1/34) of anestrus cases. Active surveillance using iELISA revealed a brucellosis incidence of 18.3% in abortion with RFM cases, 14% in RFM cases, 9.4% in repeat breeders and 0% in anestrus animals. Overall, iELISA detected anti-Brucella antibodies in 11.5% of animals exhibiting reproductive disorders, making it the most sensitive diagnostic tool among those applied. The integration of syndromic and active surveillance approaches in this study effectively identified brucellosis as a major cause of abortion associated reproductive problems in the Bidar district.

**Key words:** Brucellosis, Cattle farm, iELISA, Reproductive disorders, Syndromic surveillance.

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## INTRODUCTION

Brucellosis is a highly contagious zoonotic disease caused by Gram-negative, non-motile, non-spore-forming, aerobic, facultative intracellular coccobacilli of the genus *Brucella*. Although it primarily affects ruminants, the disease can also occur in equines, canines and felines (Suresh *et al.*, 2022). Among the 12 recognized *Brucella* species, *B. abortus*, *B. melitensis* and *B. suis* are the most significant due to their pathogenicity and impact on both animal and human health (Whatmore *et al.*, 2016). In cattle, *B. abortus* is the predominant causative agent and is responsible for considerable economic losses in many African and Asian countries where the disease remains endemic. The organism causes a range of reproductive disorders such as abortions, infertility, stillbirths, retained placenta and reduced milk yield in females, as well as orchitis in males. These reproductive inefficiencies reduce dairy productivity and negatively affect the livelihoods of farmers particularly in developing regions like India. In endemic areas nearly half of infectious abortions are attributed to pathogens including *Brucella* species (Chahota *et al.*, 2003).

The economic burden of brucellosis is substantial. Global estimates suggest annual losses exceeding US \$3 billion largely due to abortion, infertility, reduced milk output and calf mortality with per-animal losses estimated at US \$6.8 per cow and US \$18.2 per buffalo (Singh *et al.*, 2015).

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For farming households, the disease reduces productivity, increases veterinary expenses and shortens the productive

lifespan of affected animals. Beyond direct production losses, brucellosis also has implications for food safety, food security and international livestock trade (Song *et al.*, 2018; Adetunji *et al.*, 2020; Khurana *et al.*, 2021). Multiple risk factors for brucellosis have been identified and broadly classified into agro-ecological, host-related, management-related and farmer-related categories. Numerous studies have demonstrated significant associations between seropositivity and factors such as species, sex, breed and age (Chand and Chhabra, 2013; Shome *et al.*, 2014; Patel *et al.*, 2015).

Milk ring test (MRT) is widely used as a preliminary herd-level screening method typically followed by serological testing of individual animals to confirm disease status. Serological assays remain the cornerstone of brucellosis diagnosis and control (Erdenlig *et al.*, 2017; Jamil *et al.*, 2020). Commonly used tests include the Serum Agglutination test (SAT), Rose Bengal test, Complement Fixation test and ELISA. In cattle, the Rose Bengal plate test (RBPT) is recommended as an initial screening tool with positive samples confirmed using ELISA or the Complement Fixation test (Gwida *et al.*, 2010). Given the global significance of bovine brucellosis, effective control and eventual eradication depend on early, accurate diagnosis and routine herd-level surveillance. Understanding the associations between seropositivity and reproductive outcomes such as abortion and infertility supports evidence-based interventions including vaccination strategies and improved herd management practices. Hence, this study was planned to survey the infertility and abortions due to brucellosis in cattle farms in Bidar District of Karnataka

## MATERIALS AND METHODS

### Study Plan and Design

The research work was carried out at organised dairy farms in five different tehsils of Bidar district, Karnataka, India by the Department of Animal Reproduction, Gynaecology and Obstetrics of Veterinary College, Bidar (KVAFSU) in collaboration with ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru and Institute of Animal Health and Veterinary Biologicals (IAH & VB), Bengaluru, Karnataka, India. The study was conducted following approval of the Institutional Animal Ethics Committee. A total of 200 milk and blood samples were collected from various cattle farms across the Bidar district that reported reproductive disorders such as abortions, retention of fetal membranes (RFM), repeat breeding syndrome (RBS) and anestrus. A structured questionnaire was employed to gather detailed information on animal-level factors including reproductive history (abortions, RFM, RBS, anestrus), age, sex, breed and parity. In addition demographic data were recorded specifying the tehsil within Bidar district from which the sampled farms were selected.

### Processing of Milk and Serum Samples

A total of 200 animals from five organised farms in five tehsils of the Bidar district each presenting a history of late-gestation abortion, retention of fetal membranes, anestrus or repeat breeding were selected for milk and blood sampling. From each of the five farms/tehsils, 40 individual cows' milk samples along with one pooled milk sample were collected. Pooled milk samples were screened for brucellosis using the Milk ring test (MRT) following the standard protocol (Alton *et al.*, 1988) with the Abortus Bang Ring antigen sourced from the Institute of Animal Health and Veterinary Biologicals (IAH&VB), Bengaluru.

Approximately 10 mL of blood was drawn aseptically by jugular venipuncture into clot activator vacutainers and allowed to clot with careful handling to avoid haemolysis. The samples were subsequently transported to the laboratory under refrigerated conditions at 4 °C. Serum samples of all selected individual animals were subjected to preliminary screening using the Rose Bengal plate test (RBPT) as per established guidelines (Alton *et al.*, 1988) employing the *Brucella abortus* S19 coloured antigen procured from IAH&VB, Hebbal, Bengaluru. Indirect ELISA (iELISA) was carried out in accordance with the instructions provided by NIVEDI, Bengaluru. Based on the percentage positivity values relative to the positive control, samples showing <55% were classified as negative and those >65% were considered positive.

### Statistical Analysis

Significance difference of study parameters was determined by Chi-square test and p value. The statistical software SPSS, Medcalc-22.030 was used for the analysis of data. Diagnostic sensitivity (DSc), specificity (DSp), positive predictive value (PPV), negative predictive value (NPV), accuracy were determined at 95% confidence interval (95% CI) using Medcalc statistical software.

## RESULTS AND DISCUSSION

Among the recorded 200 reproductive disorders cases in dairy cows of Bidar district, RFM was the most prevalent accounting for 32.0% (64 cases) followed by repeat breeding syndrome at 26.5% (53 cases), abortion accompanied by RFM at 24.5% (49 cases) and anestrus at 17.0% (34 cases). These findings were consistent with the observations of Gujrathi (2016), who reported RFM as the most common reproductive disorder (28.57%) followed by abortion (21.42%) and equal frequencies of repeat breeding and anestrus (14.88% each). Similarly, Shantveer (2022) identified RFM as the major issue (28%) followed by repeat breeding (25%) and abortion (18%). In contrast, lower prevalence rates of RFM at 17% and 10.3%, abortions at 5.9% and 12.2%, repeat breeding at 6.2% and 10.3% and anestrus at 10.1% and 10.3% have been documented from Ethiopia by Haile *et al.* (2010) and Benti and Zewdie (2014), respectively. Patel *et al.* (2015) further reported considerably lower rates of abortion (3.31%), RFM (4.3%)



and repeat breeding (3.26%) from Brucellosis in Gujarat, while Khan *et al.* (2016) documented a higher prevalence of anestrus (31.79%) followed by repeat breeding (24.61%), RFM (14.35%) and abortions (11.25%) from North-Eastern India. Kutty and Ramachandran (2003) reported markedly higher rates of anestrus (65%) with repeat breeding incidences of 35% from Kerala, probably due to geographic and nutritional differences.

The farm-wise pooled milk samples when tested using the MRT, farms 1 and 2 yielded positive results, while farms 3, 4 and 5 tested negative (Table 1). These findings concurred with the observations of Kanaka (2009) from 8 milk unions in Tirupati, who reported MRT positivity in specific villages under union 1 and detected 5, 3 and 6 positive samples in unions 3, 6 and 7 respectively, while no positive cases were reported from other 4 unions. The positive and negative pooled samples from different farms likely indicate difference in the proportion of *Brucella*-infected cows and their antibody levels making difference in the sensitivity or threshold level of detectable antibodies into the milk enabling MRT detection. It is well documented that MRT has limitations when used on pooled milk samples due to its reduced sensitivity in detecting low antibody levels (OIE, 2009). Although MRT remains a valuable screening tool for individual cows, it may yield false positives in animals recently vaccinated (within four months), those with mastitis or cows in early lactation (Ali *et al.*, 2013; Kumar *et al.*, 2016), and in larger herds exceeding 100 lactating cows (OIE, 2009).

In the syndromic surveillance of brucellosis conducted on 200 individual cows, MRT identified brucellosis positivity in 8 of 49 (16.32 %) abortion cases with retention of fetal membranes, 4 of 64 (6.25%) RFM cases, 3 of 53 (5.66%) repeat breeder syndrome (RBS) cases and 1 of 34 (2.90%) anestrus

cases, with the overall 16 of 200 (8.0%) cases (Table 2). These findings were comparable to those of Patel *et al.* (2015), who reported positivity rates of 17.24% in abortion cases, 8.45% in RFM and 3.94% in RBS. Kumar *et al.* (2017) also documented 20.51% brucella positivity in abortions, 4.76% in RFM and 1.72% in other reproductive disorders. In contrast, higher prevalence rates were recorded by Gogoi *et al.* (2017) and Shantveer (2022) as 33.68% and 22.20% in abortions, 19.54% and 17.8% in RFM and 17.50% and 12.00% in RBS cases, respectively. Although MRT-based syndromic surveillance in this study detected positive animals among those with a history of infertility and abortions; they all can't be declared as brucellosis positive as milk ring test is primary screening test and needs to be confirmed by combination of other tests. The limited sensitivity of MRT in detecting *Brucella* infection has also been highlighted by Chand and Sharma (2004). While the milk ring test remains a simple and widely used technique for detecting and monitoring *Brucella* antibodies in bovine milk (Alton *et al.*, 1988), it is prone to false-positive reactions in animals vaccinated within the previous four months, in cows suffering from mastitis or during early lactation (Ali *et al.*, 2013; Kumar *et al.*, 2016).

In the active surveillance, serum samples from 200 cows exhibiting various reproductive disorders were tested using RBPT and iELISA. Based on RBPT results, the incidence of brucellosis was 16.33% in cows with abortion accompanied by retention of fetal membranes, 7.81% in cases of RFM, 7.55% in repeat breeder syndrome (RBS) and 0% in anestrus cases. iELISA detected slightly higher positivity rates with 18.37% in abortion with RFM, 14.06% in RFM and 9.43% in RBS, while no anestrus cases tested positive (Table 2). These findings were consistent with the work of Gujrathi (2016), who reported brucellosis incidences of 11.2% in abortion cases with RFM,

**Table 1:** Farm categorization for syndromic surveillance of brucellosis by MRT

Farm No	Talukas	No of animals in farm	No of animals sampled	History				MRT (Pooled milk)
				RFM Yes/No	RBS Yes/No	Abortion Yes/No	Anestrus Yes/No	
1	Aurad	72	40	Yes	Yes	Yes	Yes	+
2	Bidar	81	40	Yes	Yes	Yes	Yes	+
3	Bhalki	68	40	Yes	Yes	Yes	Yes	-
4	Basavakalyan	65	40	Yes	Yes	Yes	Yes	-
5	Chitaguppa	74	40	Yes	Yes	Yes	Yes	-

**Table 2:** Syndromic (MRT) and Active surveillance (RBPT, iELISA) of brucellosis in 200 cattle with history of abortion, RFM, RBS and anestrus

Reproductive problems	Samples tested	No. positive by MRT (%)	No. positive by RBPT (%)	No. positive by iELISA (%)
Abortion + RFM	49	8 (16.33)	8 (16.33)	9 (18.37)
RFM	64	4 (6.25)	5 (7.81)	9 (14.06)
RBS	53	3 (5.66)	4 (7.55)	5 (9.43)
Anestrus	34	1 (2.90)	0 (0.00)	0 (0.00)
<b>Overall</b>	<b>200</b>	<b>16 (8.00)</b>	<b>14 (7.00)</b>	<b>23 (11.50)</b>
$\chi^2$ -square test*			15.27 / p< 0.05	

8.33% in RFM and 5.55% in abortion cases. Anuradha and Ganesan (2006) and Chettri *et al.* (2015) recorded brucella positivity in 13.5% and 16.16% of RFM cases, respectively.

However, the results of this study differ from those reported by Chettri *et al.* (2015), who found markedly higher incidences of abortion (50%) and repeat breeding (21.21%). Similarly, Jain *et al.* (2019) identified *Brucella* antibodies in 45.45% of animals with a history of abortion and in 14.54% of repeat breeders. Shantveer (2022), using iELISA also documented higher positivity rates of 27.7% in abortion cases, 21.4% in RFM and 12% in repeat breeders. Patel *et al.* (2015) reported significantly higher prevalence in abortion cases (45.50%) followed by RFM (22.89%), repeat breeding (17.86%) and the lowest in metritis/endometritis (9.50%). These findings were further supported by Jain *et al.* (2019), who observed greater seropositivity among animals with a history of abortion (45.45%) relative to repeat breeders (14.54%). Bhattacharya *et al.* (2005) found abortion to be the most affected category (55%) followed by repeat breeding (24%) and RFM (20%) for brucella positivity. Panchasara (2007) similarly reported a higher prevalence of brucellosis among abortion cases in cattle in Gujarat.

The significant chi-square value (15.27,  $p < 0.05$ ) observed in the present study indicates a non-random distribution of seropositivity across reproductive disorders. This reflects a clear association between brucellosis and specific conditions particularly abortion with RFM, while RBS and anestrus exhibited minimal or no seropositivity. The high chi-square statistic and low p-value support a true etiological relationship rather than random variation. These observations are in line with Ullah *et al.* (2019), who reported that animals with a prior history of reproductive disorders especially abortion were significantly more likely to be seropositive ( $p = 0.0056$ ) with 48% of seropositive cows having experienced an abortion. Numerous studies further substantiate that brucellosis remains one of the leading infectious causes of abortion in cattle with significantly higher brucella seropositivity among animals with previous reproductive failures (Deresa *et al.*, 2020).

## CONCLUSION

The present study demonstrates that combining syndromic and active surveillance provides a robust approach for detecting brucellosis-associated reproductive disorders in cattle. Among the major reproductive problems recorded in the Bidar district, retention of fetal membranes and repeat breeding were the most frequently observed, with brucellosis emerging as a significant underlying cause, particularly in cases of abortion with retained placenta. The integrated surveillance approach adopted in this study thus proves effective in early detection and prioritization of brucellosis management efforts, contributing to improved reproductive health and productivity in cattle.

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