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SEROPREVALENCE OF BOVINE AND CAPRINE BRUCELLOSIS DETECTED BY MILK RING TEST AND INDIRECT ELISA AT HYDERABAD, SINDH, PAKISTAN

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Article Info

Abstract

Brucellosis is a major zoonotic disease that significantly affects livestock productivity and poses a serious threat to public health, particularly in developing countries where dairy farming is a primary source of income. The present study was conducted at the Veterinary Research and Diagnosis Laboratory (CVDL), Tando Jam, to determine the seroprevalence of brucellosis in buffaloes and goats of Hyderabad district, Sindh, Pakistan. A total of 420 milk samples were collected from apparently healthy and clinically suspected animals, including buffaloes (n = 240) and goats (n = 180), and screened using Milk Ring Test (MRT) and Milk indirect ELISA (i-ELISA) for the detection of anti-Brucella antibodies. The overall prevalence detected by MRT was 6.9% (29/420), while i-ELISA detected a higher prevalence of 12.4% (52/420), indicating greater sensitivity of the ELISA technique. Species-wise analysis showed comparatively higher seropositivity in buffaloes than goats. Statistical analysis revealed a highly significant association ($P < 0.01$) between abortion history and brucellosis seropositivity, whereas stillbirth showed no significant association. The findings confirm the active circulation of Brucella infection in the study area and emphasize the need for routine screening, improved biosecurity measures, and implementation of effective control and vaccination strategies.

Keywords: *Brucellosis, Brucella abortus, Brucella melitensis, Milk Ring Test, Indirect ELISA, Seroprevalence, Abortion, Buffaloes, Goats, Hyderabad Sindh.*

Introduction

Brucellosis is a highly contagious zoonotic disease caused by Gram-negative intracellular bacteria of the genus Brucella. Brucella abortus primarily affects bovines, whereas *Brucella melitensis* predominantly infects sheep and goats (Blasco & Molina-Flores, 2011; Kaleri et al., 2025ab; Godfroid et al., 2011). The disease results in abortion, retained fetal membranes, reduced milk production, infertility, weak offspring, and significant economic losses (Nicoletti, 2010; Wadood et al., 2009). Brucellosis remains endemic in many developing countries including Pakistan, where livestock farming contributes substantially to rural livelihoods. Human infection commonly occurs through direct contact with infected animals or consumption of contaminated milk and dairy products (Ali et al., 2017). Several serological tests are available for diagnosis including Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT), and Milk Ring Test (MRT) (Kaleri et al., 2026ab; Alton et al., 1988; OIE, 2009). Milk indirect ELISA (i-ELISA) has been reported to be more sensitive than MRT for detection of anti-Brucella antibodies in milk samples (Kerkhofs et al., 1990; Shafee et al., 2011). Accurate and early diagnosis is essential for controlling herd-level transmission and minimizing economic losses. In endemic regions, subclinical infections often remain undetected, facilitating silent spread within dairy herds (Kaleri et al., 2026c; Suthar et al., 2026; Godfroid et al., 2011). Furthermore, improper disposal of aborted materials and lack of routine screening increase environmental contamination and transmission risk (Nicoletti, 2010). Buffaloes and goats play a vital role in milk production in Sindh province, yet systematic milk-based surveillance data are scarce. Evaluation of reproductive risk factors such as abortion and age is crucial for understanding disease dynamics and planning control strategies (Ali et al., 2017). Despite its economic importance, limited data are available regarding milk-based seroprevalence of brucellosis in Hyderabad district, Sindh. Therefore, the present study was designed to determine the prevalence of bovine and caprine brucellosis using MRT and milk i-ELISA in Hyderabad and to evaluate associated reproductive risk factors.

Materials and Methods

Study Area

The study was conducted in Hyderabad district, Sindh, Pakistan, from March to August 2025. Samples were analyzed at Veterinary Research and Diagnosis Laboratory (CVDL), Tando Jam.

Sample Collection

A total of 420 milk samples were collected:

- Buffaloes (n=240)
- Goats (n=180)

Approximately 5 ml of milk was collected aseptically after discarding initial streams. Samples were transported under cold chain and processed within 8 hours.

Milk Ring Test (MRT)

Commercial MRT antigen (VRI Lahore) was used. One ml of milk was mixed with 30 µl antigen and incubated at 37°C for 1 hour. Appearance of blue ring indicated positive reaction.

Milk Indirect ELISA

Commercial Brucella milk i-ELISA kit was used as per manufacturer instructions. Optical density was measured at 450 nm.

Data Collection

Information recorded included:

- Age (Young vs Adult)
- History of abortion
- Stillbirth
- Herd size
- Purpose of rearing (Commercial vs Household)

Statistical Analysis

Chi-square test was applied using SPSS version 25. P-value <0.05 considered significant.

Comparative Seroprevalence of MRT and Milk i-ELISA

A total of 420 milk samples collected from buffaloes and goats were screened for anti-*Brucella* antibodies using Milk Ring Test (MRT) and milk indirect ELISA (i-ELISA). Overall, i-ELISA detected a higher seroprevalence compared to MRT. Species-wise comparison revealed that buffaloes showed higher positivity than goats, particularly when tested by i-ELISA. The detailed results are presented in Table I.

Table I. Comparative seroprevalence of MRT and milk i-ELISA in buffaloes and goats.

Species	No. Tested	MRT Positive	i-ELISA Positive
Goats	180	6.1% (11/180)	4.4% (8/180)
Buffaloes	240	7.5% (18/240)	18.3% (44/240)
Total	420	6.9% (29/420)	12.4% (52/420)

Milk i-ELISA detected higher prevalence compared to MRT.

Association of Abortion with Brucellosis

The relationship between abortion history and brucellosis seropositivity was evaluated using chi-square analysis. A highly significant association ($P < 0.01$) was observed between abortion and positive i-ELISA results. Animals with a history of abortion showed markedly higher seropositivity compared to non-aborted animals. Results are summarized in Table II.

Table II. Association of abortion with brucellosis.

Category	No.	i-ELISA Positive	%	Chi-square	P-value
Non-aborted	392	28	7.1%	21.47	0.000**
Aborted	28	24	85.7%		

Highly significant association observed.

Association of Stillbirth with Brucellosis

The association between stillbirth and brucellosis seropositivity was also analyzed. Although stillbirth cases showed comparatively higher positivity (21.4%) than normal births (11.3%), the association was statistically non-significant ($P > 0.05$). These findings are shown in Table III.

Table III. Association of still birth with brucellosis.

Category	No.	Positive	%	Chi-square	P-value
Normal birth	378	43	11.3%	2.18	0.139 NS
Stillbirth	42	9	21.4%		

No significant association observed.

Age-wise Distribution of Brucellosis

Age was evaluated as a risk factor for brucellosis. Adult animals showed significantly higher seropositivity compared to young stock. Statistical analysis confirmed significant association ($P < 0.05$). Results are presented in Table IV.

Table IV. Age-wise Distribution of brucellosis

Age Group	Positive Samples	Chi-square	P-value
Young	9	8.94	0.003*
Adult	43		

Adults showed significantly higher prevalence.

Herd Size and Brucellosis Prevalence

Herd size was also analyzed to determine its influence on brucellosis occurrence. Larger herds (>20 animals) showed higher seropositivity compared to smaller herds. Statistical analysis indicated significant association ($P < 0.05$). Details are presented in Table V.

Table V. Relationship of herd size with positive results of milk i-ELISA.

Herd Size	Positive Samples	P-value
1–10 animals	10	0.041*
11–20 animals	18	
>20 animals	24	

Larger herd size associated with increased prevalence.

DISCUSSION

The present study demonstrated that brucellosis is actively circulating among buffaloes and goats in Hyderabad district, Sindh. The overall seroprevalence detected by milk i-ELISA (12.4%) was higher than MRT (6.9%), confirming the superior sensitivity of ELISA-based assays. Similar findings have been reported where milk i-ELISA was found more sensitive than

MRT in detecting low antibody titters (Kerkhofs et al., 1990; Shafee et al., 2011). The higher detection rate in buffaloes compared to goats may be attributed to intensive dairy management practices and frequent animal movement, which enhance transmission risk. The highly significant association between abortion and brucellosis ($P < 0.01$) supports the established pathogenic role of *Brucella abortus* and *Brucella melitensis* in causing reproductive disorders (Nicoletti, 2010; Wadood et al., 2009). Abortion is considered one of the hallmark clinical signs of brucellosis and serves as a major source of environmental contamination and herd transmission (Godfroid et al., 2011). Although stillbirth cases showed higher numerical positivity, the association was statistically non-significant, suggesting that multiple etiological agents may be involved in reproductive losses in the region. Age-wise analysis revealed significantly higher prevalence in adult animals, consistent with previous studies reporting cumulative exposure risk with increasing age (Khan et al., 2018; Ali et al., 2017). Adult animals are more likely to encounter infection due to prolonged exposure, repeated breeding cycles, and greater contact with infected herd mates. Herd size was significantly associated with seropositivity, with larger herds showing higher prevalence. Increased stocking density, poor biosecurity, and frequent introduction of animals without prior screening may explain this trend. Similar observations have been reported in endemic regions where herd expansion and uncontrolled animal trade facilitate disease spread (Greiner & Gardner, 2000; Abubakar et al., 2010). Moreover, the higher prevalence detected through milk testing highlights the value of non-invasive surveillance tools in dairy herds, particularly in areas where routine blood sampling is difficult. The findings align with previous reports emphasizing that milk ELISA is suitable for herd-level screening programs in endemic settings (Nielsen, 2002; OIE, 2009). Considering the zoonotic importance of brucellosis, the persistence of infection in dairy animals poses a public health concern, especially in communities consuming raw milk and milk products (Ali et al., 2017). Therefore, integrated control measures including vaccination, routine screening, test-and-segregation policies, and farmer awareness programs are essential to reduce disease burden in Sindh province. Overall, the findings emphasize the importance of routine milk-based screening and targeted control strategies in dairy herds of the region.

CONCLUSION

The present study confirms the active presence of brucellosis among buffaloes and goats in Hyderabad district, Sindh. Milk i-ELISA proved more sensitive than MRT for detecting anti-*Brucella* antibodies. Abortion history, adult age, and larger herd size were identified as significant risk factors. These findings highlight the urgent need for regular surveillance, awareness programs, improved biosecurity measures, and implementation of effective vaccination strategies to control brucellosis in the region.

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