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Seroprevalence and Risk Factors of Bovine Brucellosis in Sindh, Pakistan

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ABSTRACT

This cross-sectional study aimed to determine the seroprevalence and associated risk factors of bovine brucellosis in Sindh, Pakistan. A total of 1000 serum samples were randomly collected from cattle across eight districts and screened using the Rose Bengal Plate Test (RBPT) followed by confirmation with an ELISA. The overall seroprevalence was 25.0% (250/1000). District-wise analysis revealed significant geographical variation, with the highest prevalence in Hyderabad (41.6%) and the lowest in Badin (14.4%). Epidemiological factors strongly influenced infection rates. A significantly higher (χ^2=55.2, p<0.001) prevalence was found in female cattle (32.3%) compared to males (11.4%). Prevalence increased markedly with age, from 6.7% in animals under two years to 45.8% in those over six years. Seasonal variation was also evident, with the highest seropositivity (33.9%) recorded during the Monsoon season (August-October). The findings demonstrate that bovine brucellosis is highly endemic in Sindh, posing a substantial economic threat to the livestock sector and a major zoonotic risk to the human population. Key risk factors include district location, female gender, older age, and the monsoon season. Targeted intervention strategies, including vaccination programs in high-prevalence districts and heightened biosecurity during high-risk seasons, are urgently recommended.

INTRODUCTION

Brucellosis is a globally recognized bacterial zoonosis of profound public health concern and significant economic impact, particularly in developing countries. Primarily caused by Brucella abortus in cattle and buffaloes, the disease results in substantial livestock production losses through abortion, infertility, and reduced milk yield, while posing a severe, flu-like illness risk to humans through direct contact with infected animals or consumption of unpasteurized dairy products (Mufinda et al., 2017; Franc et al., 2018).

In Pakistan, brucellosis remains endemic and underreported. Recent studies indicate a highly variable but concerning seroprevalence in bovines, ranging from 9% to over 30% in different agro-ecological zones, far exceeding earlier national estimates (Ali et al., 2017; Pappas et al., 2006). The epidemiology of the disease is complex, driven by a confluence of demographic, management, and geographical risk factors, including herd size, animal movement, and husbandry practices (Khan et al., 2021).

Human infection typically occurs among high-risk groups such as farmers, veterinarians, and abattoir workers who handle infected tissues, placenta, or aborted fetuses. The clinical presentation in humans is non-specific, often characterized by undulant fever, malaise, and arthralgia, which can lead to chronic, debilitating complications if untreated (Dean et al., 2012). In livestock, the hallmark clinical sign is late-term abortion, often accompanied by retained placenta and subsequent

infertility, while bulls may develop orchitis or epididymitis, further facilitating transmission (Ducrotoy et al., 2017).

Despite its known burden, region-specific and current epidemiological data for key livestock areas in Pakistan are scarce. Therefore, the present study was designed to determine the contemporary prevalence of bovine brucellosis in and around Hyderabad district using the Milk Ring Test (MRT). This investigation aims to provide updated baseline data, analyze associated herd-level risk factors, and generate essential evidence to inform targeted control and eradication strategies, ultimately contributing to improved animal health, food safety, and socioeconomic security for livestock farmers in Sindh, Pakistan.

METHODOLOGY

Study Design, Area, and Sampling

A cross-sectional study was conducted from November 2022 to October 2023 in the Sindh province of Pakistan. Eight districts (Hyderabad, Tando Allahyar, Mirpurkhas, Thatta, Badin, Matiari, Jamshoro, and T. M. Khan) were selected to represent the province's diverse agro-ecological zones. A total of 1,000 blood samples were collected from cattle using a multistage random sampling technique. First, administrative units (Talukas) were randomly selected per district. Within each Taluka, villages and individual farms were then randomly chosen. From each selected herd, a maximum of 5-10 animals were sampled. The sample size was calculated using the expected prevalence formula (Thrusfield, 2018) with a 95% confidence level and 5% desired precision, assuming an expected prevalence of 20%. Data on district, age, gender, and season of sampling were recorded on a structured questionnaire.

Laboratory Analysis at CVDL, Tandojam

All laboratory procedures were performed at the Veterinary Research and Diagnosis Centre (CVDL), Tandojam, a nationally recognized reference laboratory. Serum was separated from whole blood by centrifugation and stored at -20°C until analysis.

Primary Screening

All serum samples (n=1000) were initially screened using the Rose Bengal Plate Test (RBPT) following the standard protocol of the World Organization for Animal Health (OIE, 2021). The RBPT antigen (Institute of Animal Health, Peshawar, Pakistan) was used at the recommended dilution.

Serological Confirmation

Samples showing agglutination in the RBPT were subjected to confirmatory testing using an Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA). The commercial Brucella abortus Antibody Test Kit (ID Vet, France) was used strictly according to the manufacturer's instructions. The test's sensitivity and specificity are reported by the manufacturer to be >99% and >98%, respectively. Only samples positive on both RBPT and i-ELISA were considered seropositive for brucellosis, as recommended for diagnostic certainty in surveillance studies (Nielsen & Yu, 2010).

Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS software (Version 26.0). Descriptive statistics were used to calculate overall and stratified seroprevalence. The association between seropositivity and potential risk factors (district, age, gender, season) was analyzed using the Chi-square (χ^2) test. A p-value of <0.05 was considered statistically significant.

Ethical Approval

The study protocol was reviewed and approved by the Institutional Ethical Review Committee of CVDL, Tandojam. Verbal informed consent was obtained from all animal owners prior to sample collection.

RESULTS

District wise seroprevalance of brucellosis in Sindh

A total of 1000 serum samples from cattle were screened across eight districts of Sindh. The overall seroprevalence of brucellosis was found to be 25.00% (250/1000). District-wise analysis revealed significant variation. The highest prevalence was recorded in Hyderabad district (41.60%), followed by Jamshoro (35.20%) and Mirpurkhas (28.00%). In contrast, the districts of Badin (14.40%), Thatta (16.80%), and T. M. Khan (16.80%) exhibited the lowest prevalence rates.

Table 1

Seroprevalence of brucellosis in randomly selected cattle across eight districts of Sindh, Pakistan.

S.#.	District	# of Samples Screened	# of Positive Samples	Prevalence (%)
1.	Hyderabad	125	52	41.60
2.	Tando Allahyar	125	27	21.60
3.	Mirpurkhas	125	35	**28.00**
4.	Thatta	125	21	16.80
5.	Badin	125	18	14.40
6.	Matiari	125	32	25.60
7.	Jamshoro	125	44	35.20
8.	T. M. Khan	125	21	16.80
	Total	**1000**	**250**	**25.00**

Age-Wise Prevalence of Bovine Brucellosis

The seroprevalence of brucellosis showed a strong positive association with the age of the cattle ($\chi^2 = 78.4$, $p < 0.001$). Animals younger than 2 years had a very low prevalence (6.67%). The prevalence increased markedly in the 2-4 years age group (20.24%) and peaked in cattle older than 6 years (45.83%). The highest number of positive cases was found in the 4-6 years age group (n=98), which also had a high prevalence rate of 35.00%.

Table 2

Association between age groups and seroprevalence of brucellosis in cattle (n=1000) in Sindh, Pakistan.

S.#.	Age Group (Years)	# of Samples Screened	# of Positive Samples	Prevalence (%)
1.	< 2	180	12	6.67
2.	2 – 4	420	85	20.24
3.	4 – 6	280	98	**35.00**
4.	> 6	120	55	45.83
	Total	**1000**	**250**	**25.00**

Gender-Wise Prevalence of Bovine Brucellosis

A significantly higher ($\chi^2 = 55.2$, $p < 0.001$) seroprevalence was observed in female cattle (32.31%; 210/650)

compared to males (11.43%; 40/350). Females accounted for 84% (210/250) of all positive cases, highlighting a pronounced gender-based disparity in infection rates.

Table 3

Seroprevalence of brucellosis in female and male cattle in Sindh, Pakistan.

S. #.	Gender	# of Samples Screened	# of Positive Samples	Prevalence (%)
1.	Female	650	210	**32.31**
2.	Male	350	40	11.43
	Total	**1000**	**250**	**25.00**

Season-Wise Prevalence of Bovine Brucellosis

Seroprevalence demonstrated notable seasonal variation. The highest prevalence was recorded during the Monsoon season (August-October) at 33.93%, followed by Winter at 29.41%. The lowest rates were observed in Spring (18.00%) and Summer (20.00%). The Monsoon season, despite having the second-highest number of samples, yielded the greatest number of positive cases (n=95).

Table 4

Seasonal distribution of brucellosis seropositivity in cattle in Sindh, Pakistan.

S.#.	Season	# of Samples Screened	# of Positive Samples	Prevalence (%)
1.	Spring (Feb-Apr)	250	45	18.00
2.	Summer (May-Jul)	300	60	20.00
3.	Monsoon (Aug-Oct)	280	95	**33.93**
4.	Winter (Nov-Jan)	170	50	29.41
	Total	**1000**	**250**	**25.00**

DISCUSSION

The findings of this study reveal a critical epidemiological situation, documenting an overall 25.0% seroprevalence of bovine brucellosis in Sindh, Pakistan. This figure is alarmingly high and positions Sindh as a hyperendemic region, substantially exceeding the national average reported in recent meta-analyses, which estimate bovine brucellosis in Pakistan at 11.5-15.3% (Ahsan et al., 2017; Khan et al., 2021). The disparity underscores profound regional variations in disease burden, likely driven by distinct agro-pastoral systems, transhumance patterns, and fragmented implementation of control measures. Such a high prevalence signifies not only massive economic losses from reduced fertility, abortion storms, and diminished milk yield but also an immense, ongoing zoonotic spillover risk to the province's large rural and peri-urban human population (Ducrotoy et al., 2017; Rehman et al., 2017 ab; Soomro et al., 2014; Shumaila et al., 2019).

The pronounced district-wise heterogeneity, with prevalence ranging from 14.4% in Badin to 41.6% in Hyderabad, is a pivotal finding. This spatial clustering suggests that local risk factors supersede province-wide generalizations. High-prevalence districts like Hyderabad and Jamshoro are major dairy hubs with intensive farming, high animal density, and likely more frequent informal animal trade—all factors known to amplify transmission (Cárdenas et al., 2019). Conversely, lower prevalence in coastal districts like Badin and Thatta may relate to different livestock management or breed types. This geographical pattern aligns with the "hotspot" concept in brucellosis epidemiology, where specific socio-ecological niches sustain high transmission rates, necessitating spatially targeted interventions rather than uniform policies (Bronsvoort et al., 2021).

The stark gender disparity, with females showing a 2.8-fold higher prevalence (32.31%) than males (11.43%), is pathognomonic for Brucella abortus and reaffirms the central role of reproductive pathology in disease persistence. The female reproductive tract is the primary site for bacterial colonization and shedding, especially during parturition and abortion. This finding is consistent with global literature, including recent studies from endemic regions in Africa and Asia, which identify adult females as the core reservoir within herds (Musallam et al., 2019). The practice of retaining aborting cows for milk production, coupled with inadequate disposal of contaminated birth materials, creates a vicious cycle of environmental contamination and herd reinfection, a critical gap in biosecurity that must be addressed (Godfroid et al., 2022).

The strong positive association with age, where prevalence escalated from 6.7% in juveniles (<2 years) to 45.8% in adults (>6 years), demonstrates the cumulative risk of infection over an animal's productive life. This trend is a classic epidemiological feature, reflecting increased exposure through multiple pregnancies, communal grazing, and contact with other infected animals. Older, chronically infected cows are often asymptomatic shedders, acting as silent disseminators within herds (Li et al., 2013; Afazal, 2010; Arif et al., 2019; Bayemi et al., 2009). The low prevalence in young animals is encouraging for control, as it suggests that effective calfhood vaccination could prevent a large proportion of future infections if widely implemented.

The observed seasonal pattern, with peak seropositivity (33.93%) during the Monsoon (Aug-Oct), is highly significant for practical disease management. This peak coincides with the primary calving season in Sindh, when the frequency of parturition and abortion events is highest, leading to a massive surge in environmental bacterial load. Furthermore, the monsoon humidity may prolong bacterial survival in water and soil. Similar seasonal peaks linked to calving and climatic factors have been reported in Ethiopia and India (Corbel et al., 2019; Dadar et al., 2021; Kaleri et al., 2025; Makita et al., 2021; Pakistan Economic Survey, 2017). This seasonality provides a clear window for strategic intervention: intensive surveillance, farmer education on safe parturition management, and environmental disinfection efforts should be concentrated in the pre-monsoon and monsoon periods.

Our results must be interpreted within the One Health framework. The high prevalence in cattle directly translates to a high occupational risk for farmers, veterinarians, and milk handlers. Human brucellosis in such settings is frequently misdiagnosed as malaria or typhoid, leading to chronic morbidity (Kumbhar et al., 2019; Laghari et al., 2026; Laghari et al., 2025; Mangi et al., 2015; Dadar et al., 2021). Therefore, any control strategy must be dual-purpose: protecting animal health to

safeguard economics and protecting human health to reduce disease burden.

CONCLUSION

This study conclusively demonstrates that bovine brucellosis is a highly endemic and geographically variable disease in Sindh, with a 25% overall seroprevalence driven predominantly by female cattle, older age groups, and seasonal calving patterns. These findings urgently call for the implementation of a targeted, district-specific control strategy integrating vaccination, farmer education, and a robust One Health surveillance program to mitigate its substantial economic and public health impact.

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