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Seroprevalence and Associated Risk Factors of Bovine Brucellosis among Cattle and Buffaloes in District Khairpur, Sindh, Pakistan

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ABSTRACT

Bovine brucellosis is an important reproductive and zoonotic disease that affects livestock productivity and may also pose a health risk to people who have close contact with infected animals or their products. Although brucellosis has been reported from several regions of Pakistan, district-specific information from Khairpur, Sindh, remains limited. The present study was therefore conducted to determine the seroprevalence of bovine brucellosis and evaluate selected animal- and management-related risk factors among cattle and buffaloes in District Khairpur. A cross-sectional study was conducted in eight sampling areas of District Khairpur, namely Faiz Ganj, Gambat, Khairpur, Kingri, Kot Diji, Mirwah, Nara and Sobhodero. A total of 200 blood samples were collected, comprising 100 cattle and 100 buffaloes, with 25 samples obtained from each sampling area. Serum samples were initially screened using the Rose Bengal Plate Test (RBPT), while RBPT-positive samples were subsequently examined by enzyme-linked immunosorbent assay (ELISA) at the Sindh Institute of Animal Health, Karachi. Information regarding sex, age, abortion history, herd size, breeding method, grazing system and introduction of new animals was collected through a structured questionnaire. Associations between seropositivity and categorical variables were evaluated statistically, with $P < 0.05$ considered significant. Of the 200 animals examined, 16 (8.0%) were positive by RBPT and 13 (6.5%) were confirmed positive by ELISA. ELISA-based prevalence was 6.0% in cattle and 7.0% in buffaloes, with no significant difference between species ($P = 0.774$). Seropositive animals were detected in all eight sampling areas, with area-specific prevalence ranging from 4.0% to 8.0% ($P = 0.887$). Females showed significantly greater seropositivity than males (8.0% vs. 2.0%; $P = 0.041$). Prevalence increased with age, reaching 10.0% in animals aged ≥ 7 years ($P = 0.032$). A strong association was observed with abortion history, with prevalence of 15.0% among animals with a previous abortion history compared with 2.86% among those without such history ($P = 0.003$). Large herds showed higher seropositivity (11.67%) than small herds (2.86%; $P = 0.021$). Natural mating, communal grazing and introduction of new animals were also significantly associated with seropositivity ($P = 0.048$, 0.038 and 0.044, respectively). Bovine brucellosis was present among cattle and buffaloes in District Khairpur, with an overall ELISA-confirmed seroprevalence of 6.5%. The findings indicate that both animal-related and management-related factors contribute to the occurrence of infection. Regular surveillance, screening of newly introduced animals, improved reproductive management, safer handling of abortion materials and farmer awareness are recommended to reduce transmission and associated livestock and public-health risk.

Introduction

Brucellosis is a bacterial infectious disease of livestock and humans and remains an important cause of reproductive failure and economic loss in livestock-producing communities. The disease is caused by members of the genus *Brucella*, with *Brucella abortus* being particularly important in cattle and buffaloes. Infection may remain clinically unapparent in some animals, allowing apparently healthy animals to contribute to transmission within herds (Dadar et al., 2021).

The major production-related consequences of bovine brucellosis include abortion, retained fetal membranes, infertility, repeat breeding, stillbirth and reduced reproductive efficiency. These effects can result in increased replacement costs and reduced productivity, making brucellosis an important constraint to livestock production (Khan et al., 2020). Infected animals may also shed the organism through reproductive discharges, aborted fetuses, placental tissues and other reproductive materials, increasing the possibility of environmental contamination and transmission to susceptible animals (Dadar et al., 2021).

The epidemiology of bovine brucellosis is influenced by several factors, including animal movement, herd size, reproductive history, breeding practices, introduction of replacement animals and contact between animals from different herds. These factors can vary considerably between geographical regions and production systems, resulting in substantial differences in observed prevalence (Arif et al., 2019; Suresh et al., 2022).

Pakistan has reported bovine brucellosis from several provinces and production systems. A study in the Potohar Plateau involving 2,709 animals from 253 herds reported 6.3% animal-level RBPT seropositivity, while herd-level positivity reached 18.6%, demonstrating the importance of both animal and herd characteristics in disease epidemiology (Ali et al., 2017). Similarly, a smallholder study involving cattle and buffaloes from several regions of Pakistan identified associations between brucellosis and reproductive and management-related factors (Arif et al., 2019).

Studies from Punjab have also demonstrated considerable variation in prevalence. Ali et al. (2020) reported 3.9% RBPT and 3.3% indirect ELISA positivity among animals maintained at institutional livestock farms. In contrast, Khan et al. (2020) reported substantially higher seropositivity in bovines from selected districts of upper Punjab and confirmed *Brucella* DNA by molecular testing. These differences emphasize the importance of local epidemiological investigations.

Sindh is also affected by bovine brucellosis. In Hyderabad, Mangi et al. (2015) reported substantial seropositivity among cattle using different diagnostic tests. However, the epidemiological characteristics of Khairpur differ from those of major urban dairy centers such as Hyderabad and Karachi. Consequently, prevalence estimates from other regions cannot necessarily be applied directly to Khairpur (Jamil et al., 2021).

The lack of district-specific information from Khairpur creates a need for localized epidemiological investigation. Identification of associated risk factors is particularly important because management practices such as animal introduction, natural breeding and communal grazing may influence the likelihood of exposure. Therefore, the present study was conducted to determine the seroprevalence of bovine brucellosis among cattle and buffaloes in District Khairpur, Sindh, and to evaluate selected animal- and management-related factors associated with seropositivity.

Materials and Methods

Study area and study design

A cross-sectional epidemiological study was conducted in District Khairpur Mirs, Sindh, Pakistan. The study included livestock-producing areas representing different husbandry conditions. The investigation was designed to estimate the seroprevalence of brucellosis and assess selected risk factors among cattle and buffaloes.

Study population and sampling

A total of 200 animals were included in the study, comprising 100 cattle and 100 buffaloes. Sampling was distributed equally among eight selected areas: Faiz Ganj, Gambat, Khairpur, Kingri, Kot Diji, Mirwah, Nara and Sobhoderi. Twenty-five samples were collected from each area, giving a total of 200 samples.

Blood collection and serological testing

Blood samples were collected from the selected cattle and buffaloes and processed for serum examination. The serum samples were initially screened in the field using the Rose Bengal Plate Test (RBPT). Samples showing positive reactions were subsequently subjected to ELISA confirmation at the Sindh Institute of Animal Health, Karachi. The use of a rapid screening test followed by a more specific confirmatory serological assay was selected to improve the reliability of prevalence estimation.

RBPT is commonly used for field-level brucellosis screening because it is relatively rapid, simple and practical for epidemiological surveys, while ELISA provides an additional serological approach for confirmation (Nielsen, 2002; Khurana et al., 2021).

Risk-factor assessment

A structured questionnaire was used to obtain information on selected potential risk factors. The variables included sex, age, previous abortion history, herd size, breeding method, grazing system and introduction of new animals. These factors were selected because previous investigations have identified animal movement, reproductive history and herd-management practices as important components of bovine brucellosis epidemiology (Ali et al., 2017; Arif et al., 2019).

Statistical analysis

Data obtained from field questionnaires and laboratory testing were organized and analyzed using Microsoft Excel and Statistix statistical software. Overall, species-wise and area-wise prevalence was calculated using ELISA-positive animals as the confirmed positive group. Associations between seropositivity and categorical variables were evaluated using statistical tests, and a *P*-value below 0.05 was considered statistically significant.

Results

Overall seroprevalence

Among the 200 animals examined, 16 (8.0%) were positive by RBPT. Following confirmatory ELISA testing, 13 animals (6.5%) were classified as seropositive. Cattle had an ELISA-confirmed prevalence of 6.0% (6/100), while buffaloes had a prevalence of 7.0% (7/100). The difference between species was not statistically significant (*P* = 0.774).

Table 1: Overall seroprevalence of bovine brucellosis

Species	No. examined	RBPT positive	ELISA positive	ELISA prevalence (%)	P-value
Cattle	100	8	6	6.0	0.774
Buffaloes	100	8	7	7.0	
Total	200	16	13	6.5	

Taluka-wise distribution

Seropositive animals were detected in all eight sampling areas. ELISA-confirmed prevalence was highest at 8.0% in Gambat, Khairpur, Kot Diji, Mirwah and Nara, while Faiz Ganj, Kingri and Sobhoderro showed 4.0% prevalence. The differences among sampling areas were not statistically significant (*P* = 0.887).

Table 2: Area-wise prevalence of bovine brucellosis

Sampling area	No. examined	ELISA positive	Prevalence (%)
Faiz Ganj	25	1	4.0
Gambat	25	2	8.0
Khairpur	25	2	8.0
Kingri	25	1	4.0
Kot Diji	25	2	8.0
Mirwah	25	2	8.0
Nara	25	2	8.0
Sobhoderro	25	1	4.0
Total	200	13	6.5

Sex and age

Female animals showed considerably higher seropositivity than males. Twelve of 150 females (8.0%) were ELISA-positive compared with one of 50 males (2.0%), and the association was statistically significant (*P* = 0.041).

Age was also significantly associated with seropositivity (*P* = 0.032). Prevalence increased from 3.33% in animals aged ≤3 years to 6.25% in animals aged 4–6 years and 10.0% in animals aged ≥7 years.

Table 3: Seroprevalence according to sex and age

Variable	No. examined	ELISA positive	Prevalence (%)	P-value
Male	50	1	2.0	0.041
Female	150	12	8.0	
≤3 years	60	2	3.33	0.032
4-6 years	80	5	6.25	
≥7 years	60	6	10.0	

Management and reproductive risk factors

A clear relationship was observed between abortion history and seropositivity. Among animals with a previous abortion history, 9/60 (15.0%) were ELISA-positive compared with 4/140 (2.86%) among animals without such history ($P = 0.003$).

Seroprevalence also increased with herd size. Large herds showed 11.67% positivity compared with 5.71% in medium-sized herds and 2.86% in small herds ($P = 0.021$).

Animals maintained under natural mating systems showed higher prevalence (9.17%) than those managed through artificial insemination (2.50%; $P = 0.048$). Similarly, prevalence was higher among animals using communal grazing (8.46%) than among those using private grazing (2.86%; $P = 0.038$).

Introduction of new animals was another significant factor. Herds reporting introduction of new animals had 8.33% seropositivity compared with 3.75% among herds that had not introduced new animals ($P = 0.044$).

Table 4: Major risk factors associated with ELISA-confirmed brucellosis

Risk factor	Category	No. examined	Positive	Prevalence (%)	P-value
Abortion history	Yes	60	9	15.0	0.003
	No	140	4	2.86	
	<10	70	2	2.86	
Herd size	10-20	70	4	5.71	0.021
	>20	60	7	11.67	
Breeding method	Natural mating	120	11	9.17	0.048
	Artificial insemination	80	2	2.50	
Grazing system	Communal	130	11	8.46	0.038
	Private	70	2	2.86	
New animals introduced	Yes	120	10	8.33	0.044
	No	80	3	3.75	

Discussion

The present study confirmed the occurrence of bovine brucellosis in cattle and buffaloes in District Khairpur, with an overall ELISA-confirmed seroprevalence of 6.5%. The lower proportion of ELISA-positive animals compared with RBPT-positive animals illustrates the importance of confirmatory testing when estimating seroprevalence. Diagnostic method is an important consideration when comparing prevalence estimates between studies because different tests may vary in sensitivity, specificity and interpretation (Nielsen, 2002; Khurana et al., 2021).

The 6.5% prevalence observed in Khairpur falls within the range reported in several Pakistani studies. Ali et al. (2017) reported 6.3% animal-level RBPT positivity in the Potohar Plateau, while Ali et al. (2020) reported lower overall prevalence of 3.9% by RBPT and 3.3% by indirect ELISA in institutional livestock farms. Conversely, considerably higher prevalence has been reported in selected populations, including 16.4% RBPT and 17.7% ELISA positivity in bovines from upper Punjab (Khan et al., 2020). Such variation is likely to reflect differences in geographical location, herd structure, management practices, animal movement and diagnostic methodology rather than a uniform national prevalence (Jamil et al., 2021).

Buffaloes showed slightly higher seroprevalence than cattle in the present investigation, although the difference was not statistically significant. This finding suggests that both species were exposed to broadly similar environmental and management conditions in the study area. Comparable species-level findings have been reported in Pakistani livestock populations, although other investigations have observed either cattle or buffaloes having higher prevalence depending on the production system and study population (Ali et al., 2017; Ali et al., 2020; Khan et al., 2020).

The detection of seropositive animals in all eight sampling areas is epidemiologically important. Although the prevalence ranged from 4.0% to 8.0%, the geographical differences were not statistically significant. This relatively even distribution suggests that brucellosis was not restricted to a particular sampling area and that common husbandry practices may have contributed more to exposure than geographical location alone. Localized surveillance is therefore important because substantial differences in bovine brucellosis prevalence can occur between production systems within the same country (Jamil et al., 2021).

Female animals had significantly greater seropositivity than males. The predominance among females may partly reflect their longer retention in dairy herds and greater reproductive exposure. Females also experience repeated pregnancies and parturitions, providing more opportunities for contact with reproductive materials and environments contaminated during abortion or calving. Higher prevalence among females has also been documented in bovine brucellosis investigations in Pakistan and elsewhere (Mangi et al., 2015; Khan et al., 2020).

Age was another significant factor, with prevalence increasing from 3.33% in animals aged ≤ 3 years to 10.0% in animals aged ≥ 7 years. Older animals have generally experienced a longer period of exposure to infected animals, contaminated environments and reproductive events. Therefore, the increasing prevalence with age in the present study is biologically and epidemiologically plausible. However, age should be interpreted as an exposure-related factor rather than proof of increased intrinsic susceptibility because management and reproductive history may also change with age (Arif et al., 2019).

The strongest association in the present study was observed with abortion history. Animals with a previous abortion history had a prevalence of 15.0%, compared with only 2.86% among animals without such history. Brucellosis is well recognized as an important cause of reproductive failure, and previous Pakistani investigations have also identified abortion history as an important epidemiological indicator (Ali et al., 2017; Arif et al., 2019). Nevertheless, abortion is not specific to brucellosis and may result from numerous infectious and non-infectious causes. Therefore, abortion history should be used as a trigger for laboratory investigation rather than as a diagnostic criterion by itself.

Herd size was significantly associated with brucellosis, with prevalence increasing from 2.86% in small herds to 11.67% in large herds. Larger herds can increase the number of animals exposed to an infected individual and may also involve more animal movement, greater reproductive activity and increased environmental contamination. Similar relationships between herd size and bovine brucellosis have been reported in Pakistan (Ali et al., 2017; Arif et al., 2019).

Natural mating was associated with higher seroprevalence than artificial insemination. Animals managed under natural mating showed 9.17% positivity compared with 2.50% among animals subjected to artificial insemination. Direct contact between breeding animals may increase opportunities for exposure, particularly when breeding animals are shared among herds. Previous Pakistani research has also identified breeding or insemination practices as epidemiologically relevant factors (Ali et al., 2017; Saeed et al., 2019). However, artificial insemination should not be considered completely risk-free because its effectiveness as a control measure depends on hygienic procedures and the health status of breeding materials.

Communal grazing was also significantly associated with seropositivity. Animals using communal grazing areas had a prevalence of 8.46%, compared with 2.86% among animals using private grazing areas. Shared grazing and watering environments may increase contact between animals from different herds and can facilitate exposure to contaminated reproductive materials or environments. Similar concerns regarding animal mixing, environmental contamination and husbandry practices have been highlighted in brucellosis epidemiology (Ducrotoy et al., 2017; Dadar et al., 2021).

The introduction of new animals into herds was another significant risk factor. Animals from herds that introduced new stock had a prevalence of 8.33%, compared with 3.75% in herds that did not introduce new animals. This finding is consistent with the epidemiological principle that apparently healthy animals may introduce infection into previously unaffected herds. Screening and quarantine of replacement animals are therefore important preventive measures (Ali et al., 2017; Arif et al., 2019).

The results indicate that bovine brucellosis in Khairpur is influenced by a combination of animal characteristics and management practices. The findings are particularly relevant because the disease is zoonotic and infected reproductive materials may expose farmers, animal handlers and veterinary personnel. Control should therefore be approached from both livestock-health and public-health perspectives (Dadar et al., 2021; Jamil et al., 2021).

Conclusion

Bovine brucellosis was confirmed among cattle and buffaloes in District Khairpur, Sindh, with an overall ELISA-confirmed seroprevalence of 6.5%. Although buffaloes showed slightly higher prevalence than cattle, the species difference was not statistically significant. The infection was detected across all eight investigated sampling areas, indicating that the disease was geographically distributed within the study population. Female sex, increasing age, abortion history, larger herd size, natural

matings, communal grazing and introduction of new animals were significantly associated with seropositivity. These findings indicate that transmission in the study area is likely influenced by both animal-level exposure and herd-management practices. The results support the need for continued surveillance of cattle and buffaloes in Khairpur, particularly animals with reproductive problems and herds practicing high-risk management practices. Testing of newly introduced animals, appropriate quarantine, improved reproductive hygiene, safer disposal of aborted materials and farmer education should form important components of local brucellosis-control efforts.

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Conflict of Interest

All authors have no conflict of interest

Authors Contributions

All authors have read and agreed to the published version of the manuscript. Conceptualization: RA, QK, and MMR; Literature search and data collection: AMM, ASB, and ATS; Critical review of literature: MAJ, AAB, and ZUR; Manuscript drafting: RA; Manuscript review: ASB, QK, MMR, and AMM; Supervision: QK.

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